TESIS DOCTORAL

Greenhouse gas fluxes derived from agricultural practices in forage crops in the Atlantic area

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DEPARTAMENTO DE PRODUCCIÓN VEGETAL

ESCUELA POLITÉCNICA SUPERIOR

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Autorizan la presentación de la tesis indicada, teniendo en cuenta que cumple con los requisitos del artículo 34 del Reglamento de Estudios de Doctorado y que no incurre en las causas de abstención previstas en la Ley 30/1992.

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Fdo. María Jesús Sainz Osés

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RESUMEN

Según los datos del Grupo Intergubernamental de expertos sobre el cambio climático de las Naciones Unidas (IPCC), el sector de la agricultura, silvicultura y uso del suelo (AFOLU) es responsable de casi un cuarto (10-12 Gt CO_2 equivalentes por año) de las emisiones de gases de efecto invernadero (GEI) de carácter antropogénico: óxido nitroso (N_2O), metano (CH_4) y dióxido de carbono (CO_2). Este hecho es debido principalmente a la deforestación, a las pérdidas procedentes de los suelos debidas al ciclo de nutrientes y al manejo del ganado. Las pérdidas de gases procedentes de las prácticas agrícolas en explotaciones de leche están siendo objeto de estudio en todo el mundo. En concreto, en Europa, el impacto ambiental derivado de la producción de leche está ampliamente documentado y se están adoptando políticas para mitigar y reducir estas pérdidas. Sin embargo, hasta la fecha, el impacto del sector lácteo sobre las pérdidas de GEI en el noroeste de España, donde la producción de leche es una actividad relevante, es todavía bastante desconocido. Es importante abordar esta laguna de conocimiento debido a la gran influencia que los factores edafoclimáticos tienen en la magnitud y variabilidad espacial y temporal de los flujos de GEI, y que varían enormemente de una región a otra.

El objetivo general de este trabajo fue evaluar el impacto de ciertas prácticas agrícolas utilizadas en la producción lechera sobre las pérdidas de GEI procedentes de los suelos en las condiciones climáticas atlánticas de Galicia (noroeste de España), para finalmente, obtener recomendaciones de prácticas que reduzcan las emisiones y aumenten los rendimientos de los cultivos forrajeros. Para abordar este objetivo principal, se establecieron una serie de objetivos específicos a través de cinco estudios de experimentación en parcela. Estos objetivos fueron los siguientes:

- Desarrollar modelos empíricos que expliquen las variaciones anuales y estacionales procedentes de praderas aprovechadas por pastoreo con ganado vacuno y fertilizadas, basándose en la medida de aquellos parámetros que mayor influencia puedan tener en las emisiones de N_2O.

- Cuantificar los efectos de la aplicación de fertilizantes minerales y purín de vacuno en la primavera, el verano y el otoño sobre los GEI procedentes de una pradera aprovechada por pastoreo.

- Conocer el efecto del manejo habitual del purín y del fertilizante mineral en las emisiones de GEI en un suelo de pradera del suroeste de Inglaterra.

- Entender la dinámica de los flujos de N_2O durante el cultivo del maíz forrajero bajo las condiciones climáticas gallegas, determinando la influencia del tipo de fertilizantes (purines inyectados, fertilizante mineral) comúnmente aplicados por los productores gallegos en las emisiones resultantes.

- Investigar la contribución de los diferentes fertilizantes nitrogenados, tradicionalmente utilizados en Galicia para el cultivo del maíz forrajero en los flujos de CH_4, CO_2 y N_2O en un período a corto plazo tras su aplicación, cuantificar los flujos totales de N_2O durante el todo el período de crecimiento del cultivo y aportar factores de emisión en función a los rendimientos obtenidos ('yield scaled N_2O emission factors').

Cuatro de estos estudios se llevaron a cabo entre 2007-2011 en el Centro de Investigaciones Agrarias de Mabegondo (CIAM-INGACAL) en Galicia. El quinto fue desarrollado durante 2011 en Rothamsted Research North Wyke, en Devon (suroeste de Inglaterra), si bien, en un tipo de suelo y manejo de la pradera diferentes al de Galicia, bajo condiciones climáticas similares.

En el primer estudio (CIAM-INGACAL, Galicia) el objetivo fue desarrollar modelos empíricos para explicar las variaciones interanuales y estacionales de las pérdidas de N_2O procedentes de praderas fertilizadas y pastadas mediante la medida de los parámetros de mayor influencia. Para ello se monitorizaron los flujos de N_2O en seis praderas con diferentes manejos de pastoreo y fertilización mineral y los de una pradera control (CN) (sin aportes de N procedentes del pastoreo o fertilización), entre los meses de abril del 2007 y diciembre del 2009 usando la técnica de la cámara cerrada. El primer modelo se desarrolló para predecir las variaciones del N_2O emitido a escala anual ('interanual') y los otros cuatro a nivel estacional (invierno: 'Jan-Mar', primavera: 'Apr-Jun', verano: 'Jul-Sep' y otoño: 'Oct-Dec'). Los cinco modelos empíricos obtenidos se basaron en las ecuaciones que mejor se ajustaban (Adj R^2) a los siguientes parámetros (predictores): precipitación, temperatura media del aire y del suelo, media de porcentaje de poros del suelo llenos de agua (WFPS), media del contenido de amonio (NH_4^+-N) y nitrato (NO_3^--N) en la capa superficial (0-10 cm) del suelo, cantidad de fertilizante nitrogenado (N*fert*) y N excretado por el ganado (N*excret*). Para el modelo 'interanual', se consideraron los datos correspondientes entre enero 2008 y diciembre 2009. La media anual de la emisión de N_2O (kg ha^{-1}) procedente de las praderas con manejo de N (pastoreo y fertilización mineral) se encontró en el rango 22,2-30,1 kg N ha^{-1} y en el rango 12,5-13,6 kg N ha^{-1} en la pradera CN. El modelo 'interanual' mostró que el 24% de la variación en la cantidad de N_2O emitido fue causada por la media de WFPS en el suelo, la precipitación, el N*fert* y el N*excret* (P<0,001). Otros factores no incluidos en el modelo y probablemente relacionados con el pastoreo (p.ej. cambios en la densidad aparente, pH, contenidos de carbono (C) disponible) pudieron haber sido los responsables de la variación no explicada en el modelo. No se obtuvo un modelo significativo para el periodo 'Jan-

Mar' (P=0,077), probablemente debido a la menor frecuencia de muestreo de gases en esta estación durante el 2008. Sin embargo, si fueron significativos para el resto de las estaciones. Para los modelos 'Apr-Jun', 'Jul-Sep' y 'Oct-Dec', fueron incluidos los datos correspondientes a cada periodo en 2007, 2008 y 2009, obteniendo variaciones en la emisión acumulada de N_2O de hasta 3,5, 2,7 y 2,0 en el periodo 'Apr-Jun', 'Jul-Sep' y 'Oct-Dec', respectivamente. En 'Apr-Jun', el 77% de la variación fue causada por el efecto combinado de la temperatura media del suelo y del N*fert* (P<0,001). En el periodo 'Jul-Sep', la combinación de la temperatura media del suelo y del contenido medio de NO_3^--N en el suelo explicaron el 72% de la variación (P<0,001). Para el periodo 'Oct-Dec', el 78.4% de la variación fue debida a las diferencias en la media de WFPS del suelo, en la precipitación, en el contenido medio de NH_4^+-N en el suelo y en el N*excret* (P<0,001). Con el objetivo de reducir las emisiones anuales de N_2O procedentes de praderas destinadas a la producción lechera en el noroeste de España, se sugiere una restricción del número de horas de pastoreo al día, así como evitar el pastoreo cuando el suelo está muy húmedo, especialmente durante el otoño. Así mismo, se destaca la necesidad de llevar a cabo más investigaciones de campo para la obtención de modelos que estén basados en datos más detallados relacionados con el pastoreo y que permitan una mejor predicción de las variaciones interanuales del N_2O.

En el segundo estudio (CIAM-INGACAL, Galicia), el objetivo fue cuantificar los efectos de la aplicación de fertilizantes minerales y purín de vacuno en las emisiones de GEI procedentes de praderas pastadas por vacuno de leche así como identificar el tipo de fertilizante que sin elevar los flujos de GEI incrementa la producción. Los flujos de GEI fueron monitorizados entre abril y diciembre del 2011 tras las aplicaciones en primavera, verano y otoño de un fertilizante mineral (MN) así como tras la inyección de purín de vacuno (CS). Además se añadió un tratamiento control sin fertilización nitrogenada (CN). Las dosis aplicadas en el tratamiento MN fueron de 36, 45 y 40 kg N ha^{-1} en primavera, verano y otoño, respectivamente. En el tratamiento CS, se trató de igualar en cada fertilización la cantidad de N mineral contenido en el purín de vacuno al aplicado en el tratamiento MN. Teniendo en cuenta esto, 26, 35 y 28 m^3 ha^{-1} de purín de vacuno se inyectaron a 5 cm de profundidad ('shallow injection') en la primavera, el verano y el otoño, respectivamente. Durante el año 2011, se produjo una reducción de la precipitación media, especialmente importante durante los periodos de primavera y verano, causando en el suelo una disminución del WFPS (<60%WFPS). Estas condiciones de sequía contrastaron con los valores de WFPS (>60%WFPS) observados durante el otoño debido a la mayor frecuencia en las precipitaciones. Las pérdidas totales acumuladas de N_2O fueron similares en los tratamientos CS y MN (P>0,05), produciéndose las más elevadas durante los 30 primeros días tras la aplicación de primavera, debido a la coincidencia de precipitaciones tras la aplicación de los fertilizantes así como tras la aplicación de otoño. Se observaron flujos de CH_4 de elevada magnitud inmediatamente después de cada inyección de purín, los cuáles disminuyeron en los días sucesivos hasta alcanzar valores similares a los tratamientos CN y MN. Los valores totales

acumulados de CH_4 fueron más elevados en el tratamiento CS que en CN y MN (P<0,05). Los mayores flujos de CH_4 procedentes del tratamiento CS fueron observados tras la aplicación en otoño (P<0,05). Este resultado pudo deberse al menor contenido en materia seca del purín usado en la aplicación del otoño, en comparación con el usado en la aplicación de la primavera, lo cual habría facilitado su infiltración en el suelo y la generación de condiciones más favorables para el proceso metanogénico. Sin embargo, el contenido en materia seca del purín empleado en la aplicación del verano fue similar al del otoño, lo cual denotaría que el consumo de CH_4 en el suelo en el otoño pudo estar condicionado por el estado anaeróbico del suelo, incrementando así la cantidad de CH_4 liberado tras la aplicación del purín. También se observaron elevados flujos de CO_2 tras cada inyección de purín y, al igual que el CH_4, de corta duración, disminuyendo en los días posteriores a cada aplicación hasta alcanzar valores similares a los obtenidos con los tratamientos CN y MN. Sin embargo, a pesar de estos flujos elevados de CO_2, no se encontraron diferencias significativas en las pérdidas totales acumuladas de CO_2 respecto a los tratamientos MN y CN (P>0,05). En general, no se encontraron diferencias significativas entre tratamientos cuando el total de pérdidas de GEI (suma de N_2O, CH_4 y CO_2) se expresó en equivalentes de CO_2 (CO_2-C eq) (P>0,05). Los valores obtenidos se encontraron en el rango 3,87-4,52 Mg CO_2-C eq ha^{-1}. Los factores de emisión en función del rendimiento en materia seca (DM) obtenido ('yield scaled emission factors') fueron de 3,87 Mg CO_2-C eq Mg DM^{-1} para el tratamiento CN, valor similar a los 4,41 y 4,52 Mg CO_2-C eq Mg DM^{-1} obtenidos con los tratamientos MN y CS, respectivamente (P>0,05). Las condiciones de sequía del suelo durante la primavera y el verano podrían haber estimulado la mineralización de la materia orgánica del suelo, aportando cantidades de N suficientes para cubrir las demandas de la pradera. Como ambos fertilizantes liberaron cantidades similares totales de equivalentes de CO_2 para obtener los mismos rendimientos, no hubo un beneficio ambiental por la utilización de uno u otro. Sin embargo, si se considera el coste de la compra de fertilizantes minerales, la inyección de purines de vacuno podría ser más beneficiosa para los productores de leche ya que las deyecciones ganaderas en las explotaciones podrían ser reciclados así como se reducirían los costes derivados de la producción de leche.

En el tercer estudio (Rothamsted Research North Wyke, Inglaterra), el objetivo fue evaluar como la aplicación de purín de vacuno (SL) o fertilizante mineral (AN) en primavera, verano y otoño afectaba a la emisión de GEI procedente de un suelo de pradera. El experimento se llevo a cabo entre mayo y noviembre del 2011 en el sureste de Inglaterra, una región caracterizada por una elevada precipitación anual (>1000 mm $año^{-1}$) y por suaves temperaturas (temperatura media anual del aire de 9,6 $^{\circ}$C). Los tratamientos MN y SL fueron aplicados en mayo (primera aplicación), junio (segunda aplicación) y septiembre (tercera aplicación) con dosis de 80 kg N ha^{-1} en cada aplicación. Las condiciones de sequía observadas durante la primavera y el verano (inusuales de la zona de estudio) y las precipitaciones recogidas en el otoño afectaron al WFPS del suelo resultando en bajos flujos de N_2O

durante el estudio. Tras las primeras dos aplicaciones (en primavera y verano), las condiciones climáticas disminuyeron los valores de humedad del suelo por debajo del 60%WFPS, el valor umbral para las pérdidas de N_2O por nitrificación. Por el contrario, las precipitaciones frecuentes tras la tercera aplicación (en otoño) ocasionaron un incremento de los valores de WFPS y estimularon las pérdidas de N_2O por desnitrificación. Respecto al tipo de fertilizante, AN causó mayores emisiones acumuladas de N_2O en comparación con el SL tras la tercera aplicación (P<0,05), probablemente debidas a las mayores condiciones de anaerobiosis en el suelo en este tratamiento y a la volatilización del amoniaco (NH_3), la cual redujo la cantidad de N mineral en el suelo disponible para la producción y emisión de N_2O. El modelo de regresión múltiple 'Ammonia Loss from Field Applied Manure' (ALFAM) estimó las pérdidas de N por volatilización de NH_3 entre el 25 y el 38% del N aplicado como consecuencia de la distribución del purín en superficie ('surface broadcast'). La extracción de N por la planta representó cerca del total del N aplicado en el tratamiento AN tras las dos primeras aplicaciones y el 59% tras la tercera aplicación, mientras que en el tratamiento SL la extracción de N por la planta supuso una media del 64% del total de N aplicado. Los flujos de nitrógeno molecular (N_2) no fueron medidos. Sin embargo, las precipitaciones registradas tras la tercera fertilización incrementaron gradualmente los contenidos de WFPS en el suelo hasta la saturación pudiendo haber ocasionado pérdidas de N debidas a una completa desnitrificación, especialmente en el tratamiento AN. Así, las aplicaciones de AN y SL resultaron en pérdidas totales de N_2O durante los 6 meses muestreados de 0,21 y 0,17 kg N ha^{-1}, respectivamente (representando el 0,020 y 0,003% del N aplicado). Se observó producción de CH_4 en los primeros dos o tres días tras las aplicaciones del tratamiento SL. En el resto de los periodos considerados, el suelo tratado con SL actuó como un sumidero de CH_4, igual que en el tratamiento AN. Se obtuvieron valores totales netos acumulados de -0,09 y 0,92 kg CH_4 ha^{-1} para los tratamientos AN y SL, respectivamente. Las tasas de producción y consumo de CH_4 estuvieron relacionadas con los cambios en el %WFPS. Así, bajo condiciones de sequía (por debajo de 60%WFPS) el consumo de CH_4 estuvo estimulado, tal y como se observó en mayo y junio en el tratamiento AN, reduciéndose así mismo la tasa de producción en el tratamiento SL. Los valores netos acumulados de CO_2 obtenidos durante los seis meses muestreados fueron de 1,24 Mg CO_2-C ha^{-1} en el tratamiento AN y 0,35 Mg CO_2-C ha^{-1} en el tratamiento SL.

En el cuarto estudio (CIAM-INGACAL, Galicia), el objetivo fue cuantificar las emisiones de N_2O procedentes de las prácticas de fertilización llevadas a cabo por los productores gallegos de maíz forrajero (*Zea mays* L.). El maíz forrajero se cultivó durante los años 2008 y 2009, en emplazamientos diferentes dentro de la finca experimental. Los flujos de N_2O fueron medidos durante todo el periodo de crecimiento del cultivo tras la aplicación de los siguientes tratamientos: cultivo sin aplicación de N (CN); con 200 kg N ha^{-1} de fertilizante mineral (MN); con 200 kg N ha^{-1} de purín de vacuno inyectado (CS); con 200 kg N ha^{-1} de purín de porcino inyectado (PS). El fertilizante mineral se aplicó en dos dosis: 125 kg N ha^{-1} en forma de fertilizante NPK en siembra y 75 kg N ha^{-1} de urea granulada en la

cobertera. Los purines de vacuno y porcino fueron aplicados en su totalidad en el momento previo a la siembra. Se observó que a pesar de que la fertilización incrementó de manera significativa las pérdidas de N en forma de N_2O, el tipo de fertilizante no afectó de manera significativa las emisiones totales acumuladas en ninguno de los años estudiados (P>0,05). Esto podría deberse al elevado contenido de C existente en el suelo de ambos emplazamientos. Las emisiones totales acumuladas de N_2O procedentes de los tratamientos con aplicación de N (MN, CS y PS) se encontraron en los rangos 19,8-20,5 kg N ha^{-1} en 2008 y 10,8-11,7 kg N ha^{-1} en 2009, siendo el periodo entre la siembra y la cobertera el de mayor contribución a las emisiones totales resultantes del cultivo de maíz forrajero. La nitrificación probablemente causó los flujos de N_2O observados durante los días siguientes a la aplicación de N en el momento de la siembra (mayo), sin embargo, las mayores pérdidas de N_2O fueron observadas bajo óptimas condiciones para la desnitrificación. Las variaciones en los flujos de N_2O entre estaciones de cultivo podrían atribuirse, en primer lugar, a una mayor producción de N_2 durante 2009 como consecuencia de los contenidos de WFPS más elevados (>80%WFPS) durante la mayor parte del periodo muestreado en 2009. Las precipitaciones que humedecieron el suelo tras periodos de sequía acontecidos durante el periodo de cultivo en 2008, ocasionaron los mayores flujos de N_2O observados, y contribuyeron a incrementar aproximadamente un 40% el total de las emisiones de N_2O durante este año. Con el uso de purines o fertilizantes minerales se obtuvieron similares perdidas de N_2O para la obtención de los mismos rendimientos ('yield scaled emission factors') (P>0,05), con valores tras la aplicación de 200 kg N ha^{-1} en el rango 1,18-1,23 kg N_2O-N Mg^{-1} DM en 2008 y 0,51-0,58 kg N_2O-N Mg^{-1} DM en 2009. Estos resultados coinciden con los obtenidos en el quinto estudio (CIAM-INGACAL, Galicia), durante el periodo de cultivo de maíz forrajero en 2010, donde también se cuantificaron las emisiones totales de N_2O durante el periodo de crecimiento del cultivo y se obtuvieron factores de emisión en función de los rendimientos alcanzados tras la aplicación de los mismos tipos de fertilizantes nitrogenados. En este estudio, al igual que en el anterior, la aplicación de fertilizantes nitrogenados incrementó de forma significativa las pérdidas de N_2O, especialmente entre la siembra y la cobertera (P<0,05), con valores de potencial de calentamiento global (GWP) para el N_2O de 1,56 Mg CO_2 eq ha^{-1} en el tratamiento CN (sin adición de N) y entre 2,33-2,61 Mg CO_2 eq ha^{-1} para los distintos tratamientos con fertilización nitrogenada. No existieron diferencias significativas entre los valores de GWP para el N_2O entre los tratamientos con aportación de N (P>0,05), probablemente debido al elevado contenido inicial de N mineral del suelo y las elevadas tasas de mineralización observadas. Como resultado, los fertilizantes no incrementaron la producción de maíz forrajero, con recuperaciones medias aparentes de nitrógeno (ANR) del 4,29% (rango entre -3,26 y 11,7%) y 'yield scaled emission factors', expresados como cantidad de N extraído, de 36,8 g N_2O kg N^{-1} en el tratamiento CN y entre 53,0 y 57,9 g N_2O kg N^{-1} en los tratamientos con fertilización nitrogenada.

En este estudio también se investigó el uso de los diferentes tipos de fertilizantes en los flujos de CH_4, CO_2 y N_2O en un periodo a corto plazo, que comprendió el día de la aplicación de los tratamientos y los 8 días sucesivos. Durante este periodo, los mayores valores GWP (suma de N_2O, CH_4 y CO_2) fueron los obtenidos con los tratamiento PS (0,91 Mg CO_2-C eq ha^{-1}) y CS (0,71 Mg CO_2-C eq ha^{-1}) (P>0,05). Esto fue debido a los flujos de CH_4 y CO_2 observados inmediatamente después de la inyección en el suelo en estos tratamientos. Por otro lado, el tratamiento MN resulto en similares valores de GWP (0,33 Mg CO_2-C eq ha^{-1}) que en el tratamiento sin aplicación nitrogenada (P>0,05). Sin embargo, los valores de GWP obtenidos para los tratamientos PS y CS durante el periodo a corto plazo deben considerarse con precaución ya que la longitud del periodo de muestreo pudo haber sobrestimado el efecto real de estos tratamientos.

Los cinco estudios planteados en esta tesis permiten extraer las siguientes conclusiones generales:

1. Se observaron grandes variaciones interanuales y estacionales en las emisiones de N_2O procedentes de praderas fertilizadas con fertilizante mineral y aprovechadas por pastoreo con ganado vacuno de leche. Estas pérdidas estuvieron condicionadas por las variables climáticas y de suelo, pero también por las diferencias en el manejo del N. Bajo un escenario de futuro cambio climático en Galicia, previsto por un modelo climático global, una mejora en la gestión del pastoreo podría ayudar a reducir las pérdidas anuales de N_2O derivadas de esta práctica. Los episodios de sequía seguidos de precipitaciones que humedecen el suelo pueden contribuir de manera significativa a incrementar las emisiones anuales de N_2O de los suelos y, por lo tanto, este hecho justifica seguir investigando en la identificación de los mecanismos que producen las emisiones del gas en los suelos gallegos.

2. En praderas, la aplicación de purín de vacuno como fertilizante no aumentó de forma significativa las emisiones de N_2O con respecto a la fertilización mineral. Conclusiones similares se obtuvieron durante el cultivo de maíz forrajero cuando se compararon fertilizantes orgánicos (purín de vacuno y porcino inyectados) con la fertilización mineral. Los suelos utilizados en los experimentos eran ricos en carbono por lo que este factor no limitó las emisiones de N_2O, y los elevados contenidos de N mineral en suelo favorecieron las pérdidas de este gas.

3. La modelización del efecto de la técnica de aplicación de purines en praderas mostró que una gran proporción del N mineral del purín se pierde por volatilización del amoníaco cuando el purín se distribuye en superficie en comparación con la inyección. Cuando se aplican purines como fertilizantes, la inyección superficial es recomendable en lugar de la distribución en superficie con el fin de mitigar las pérdidas indirectas de N_2O y aumentar la fracción de N mineral disponible para la planta.

4. En cuanto a los factores de emisión, la fracción de N perdido en forma de N_2O fue inferior al 1% propuesto por el IPCC cuando la aplicación de fertilizantes se llevó a cabo en condiciones de sequía y cuando el N mineral en suelo fue factor limitante. Sin embargo, en condiciones climáticas más típicas

de la zona Atlántica, que propiciaron valores de WFPS óptimos para la desnitrificación, las pérdidas de N_2O alcanzaron valores superiores al 1%, viéndose especialmente favorecidas por las elevadas cantidades de N mineral en suelo.

5. Los suelos de praderas en la zona Atlántica actuaron como sumideros de CH_4 y las aplicaciones de fertilizantes minerales no modificaron la capacidad del suelo para la captación de CH_4. Conclusiones similares se obtuvieron con la aplicación de purines si no se consideran las emisiones de CH_4 producidas inmediatamente tras la aplicación y que fueron atribuidas a la liberación del CH_4 disuelto en el purín. Esto denota que las aplicaciones de N en suelos que recibieron N durante años no modifican la actividad metanotrófica de los microorganismos presentes en dichos suelos.

6. La aplicación de purines no causó un efecto general en la respiración del ecosistema en comparación con los suelos de pradera no fertilizados o con aplicación de fertilizante mineral, incluso cuando se consideraron las elevadas emisiones de CO_2 producidas inmediatamente después de la aplicación del purín. En los suelos de praderas, fertilizantes minerales y purines generaron similares emisiones equivalentes de CO_2 para la obtención de rendimientos de cosechas semejantes, por lo que ambos fertilizantes podrían ser recomendados. Sin embargo, si se considera el coste de la compra de fertilizantes, el uso de purines inyectados como fertilizante sería más beneficioso para los productores de leche ya que las deyecciones generadas en las granjas se reciclarían y los costes de producción de leche se reducirían.

7. En suelos con maíz forrajero, fertilizantes orgánicos y minerales también propiciaron emisiones similares de N_2O para la obtención de los mismos rendimientos de cultivo (expresado por unidad de materia seca o N extraído). Sin embargo, no lograron aumentar de manera eficiente los rendimientos del cultivo y causaron grandes pérdidas de N_2O. Por ello es necesario adaptar las dosis de N a las demandas del cultivo, considerando el contenido inicial de N mineral en el suelo en el momento de la aplicación del N así como la dinámica de la mineralización de la materia orgánica del suelo, especialmente en el período entre la siembra y la aplicación de cobertera donde la demanda del N por el cultivo es menor.

ABSTRACT

Losses of nitrous oxide (N_2O), methane (CH_4) and carbon dioxide (CO_2), referred to as 'greenhouse gases (GHGs), derived from agricultural practices in dairy farms have been largely documented in Europe and policies have been adopted to mitigate and reduce those losses, especially in those regions located in the Atlantic area where this sector has greater importance. However, to date, the impact of dairy farming on losses of GHG in the NW Spain, where dairy farming is an important sector, is still unknown. The overall aim of this thesis was to evaluate the impact of the agricultural practices for dairy farming on losses of GHG from soils under the Atlantic climatic conditions in Galicia (NW Spain), in order to make recommendations for practices that reduce emissions and increase crop yields. Large inter-annual and seasonal variations in the N_2O emissions from grasslands with dairy cattle grazing management and mineral fertilization were observed. These losses were driven by climatic and soil variables but also affected by differences in N management. Under a scenario of future climate change in Galicia, predicted by a global climatic model, better grazing management could help to reduce annual N_2O losses derived from N-management. Dry-wetting episodes can significantly contribute to increased annual N_2O emissions from soils and therefore justifies further research to indentify the exact mechanisms in Galician soils. In grasslands, the use of cattle slurry as fertilizer did not significantly increase N_2O emissions with respect to mineral fertilization. Similar conclusions were obtained during forage maize cropping when organic fertilizers (injected cattle and pig slurries) were compared with mineral fertilization. The soils used in these experiments were C-rich so this factor did not limit N_2O production, and the large soil mineral N contents stimulated the losses of this gas. Modelling the effect of slurry application technique on emissions from grasslands showed that large proportions of mineral N are lost from slurries by ammonia volatilization when surface broadcast compared to when injected. When using slurries as fertilizers, shallow injection is recommended rather than surface broadcast application in order to mitigate indirect losses of N_2O and increase the fraction of mineral N available for plant N uptake. In terms of emission factors, the fraction of N lost as N_2O was lower than 1% proposed by IPCC when fertilizer applications coincided with dry weather conditions and mineral N was limited in soils. However, under the typical climatic conditions of the Atlantic area, that led optimal WFPS values for denitrification, losses of N_2O derived from fertilization can reach values beyond 1%, especially when soil mineral N levels are large. Grassland soils in the Atlantic area were sinks of CH_4 and mineral fertilizer applications did not modify soil capacity to CH_4 uptake. Similar conclusions were obtained for slurry applications, if the CH_4 emissions observed immediately after slurry application that resulted from the release of the dissolved CH_4 in the slurry were not considered. That denoted that the N applications to soils that received N for many years did not modify methanotrophy activity of the microorganisms present in those soils. Slurry applications did not cause an overall effect in the ecosystem respiration compared

to non-treated or mineral fertilized grassland soils even when the resulting high CO_2 emissions observed immediately after slurry applications were considered. In grassland soils, mineral fertilizers and cattle slurries caused similar total CO_2 equivalents to produce the same yields, so both fertilizers could be used. However, if the costs of purchasing mineral fertilizers is considered, using injected slurries as fertilizer would be more beneficial for dairy farmers as animal wastes produced on farms would be recycled and milk production costs would be reduced. In forage maize soils, organic and mineral fertilizers resulted in similar yield-scaled N_2O emissions (expressed as dry matter or N uptake) . However, they failed to efficiently increase crop yields and caused high losses of N_2O. Thus, the initial soil N contents at the moment of the N application and the dynamics of soil organic matter mineralization must be considered to adapt N rates to efficiently meet crop demands, especially in the period between sowing and top dressing application when demands are small.

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CHAPTER 1. GENERAL INTRODUCTION

1.1. The greenhouse effect and climate change

The greenhouse effect and climate change are pressing modern environmental issues. Regularly we read or listen in the news that concentrations of greenhouse gases in the atmosphere are rising, the Earth is rapidly warming and its climate is changing. According to the European Environment Agency (EEA, 2012), the consequence of these effects may lead to an increase of 2.5-4° C in the European land temperature in the later part of the 21st Century relative to the 1961-1990 average. There will be more frequent heat waves, variations in precipitation (increase in the northern European countries and decrease in the southern ones) causing river floods or droughts, and increased warming in the Arctic and the melting of the Greenland ice sheet. Global sea levels in the 21st century would also rise more than 1.7 mm a year observed over the 20th century. The report also indicates that climate change will cause human health effects (i.e. transmission of certain diseases) and changes in plant and animal characteristics or even the extinction of some species.

The greenhouse effect is a natural effect. Three primary gases make up 99.9% by volume of the Earth's atmosphere: nitrogen (N_2:77.6%), oxygen (O_2: 20.9%) and argon (Ar: 0.93%). However, it is the rare trace gases, carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), hydrofluorocarbons (HFCs), perfluorocarbons (PFCs) and sulfur hexafluoride (SF_6) that have the greatest effect on our climate and they are referred to as 'greenhouse gases (GHGs). They are important as they influence the radiation balance or neat heat balance of the Earth. When incoming solar radiation passes through the atmosphere, particles and gases absorb energy. Each particle or gas absorbs energy within a specific wavelength region. A large percentage of the incoming solar radiation is in the visible region. Greenhouse gases such as water vapour, CO_2 and CH_4 have low absorption in this region and allow most of the visible light to reach the Earth's surface. After absorption at the Earth's surface, visible energy is transformed and radiated back in the far-infrared (heat) region of the spectrum. The transparency of the atmosphere to outgoing far-infrared radiation determines how much heat can escape from the Earth and how much is trapped. The important feature of the greenhouse gases is that they absorb strongly in the far-infrared and trap heat in the troposphere and stop it from escaping to space. So without the greenhouse warming effect of the atmosphere, the Earth's average surface would be about -20°C (-4 °F) instead of 15°C (-59°F) so the balance between the incoming solar radiation and the amount of GHGs in the atmosphere is important to maintain the Earth's temperature (Hardy, 2003).

Human-induced changes in the atmospheric concentrations of these gases have occurred over recent decades. In relation to the GHGs that this thesis focuses on (CO_2, CH_4 and N_2O), the Intergovernmental Panel on Climate Change (IPCC) communicated in the last report released in 2014

that despite policies adopted by institutions and countries to mitigate GHG emissions, the increase in the atmospheric concentration of these gases has been noticeable from 2000 to 2010 (annual average of 2.2% per year) compared to the previous three decades (annual average of 1.3%) (Figure 1.1). The increase of 86% in worldwide population in the period 1970-2000 (from 3.7 to 6.9 billion) and the recent economic growth are thought to be the main drivers of the change in the atmospheric composition (IPCC, 2014).

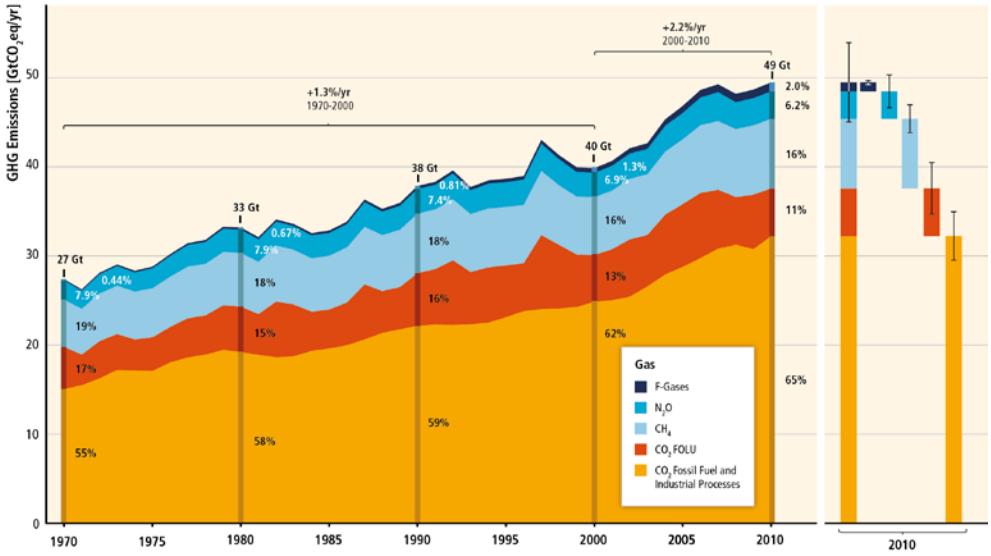

Figure 1.1. Total anthropogenic GHG emissions ($GtCO_2$ eq yr^{-1}) by groups of gases from 1970 to 2010: CO_2 from fossil fuel combustion and industrial processes; CO_2 FOLU (from forestry and other land use), CH_4 (methane), N_2O (nitrous oxide), F-gases (fluorinated gases under the Kyoto Protocol). At the right side, GHG in 2010 were broken down into these components with error bars (90% confidence interval). Emissions were converted into CO_2-equivalents based on Global Warming Potentials with a 100 year time horizon. Source: IPCC (2014).

The effect of each gas on climate change depends on several factors. The first one is the abundance or concentration of each gas in the atmosphere. According to the last IPCC report the increase of the GHG atmospheric composition observed in 2010 is mainly attributed to CO_2 (76%), followed by CH_4 (16%) and N_2O (6.2%) (Figure 1.1). The second one is their life-time in the atmosphere. The value for CO_2 is variable, contrary to CH_4 and N_2O which have atmospheric lifetimes of 12.4 and 114 years, respectively. The third factor is the Global Warming Potential (GWP), a measure of how much heat a greenhouse gas can absorb over a given period of time. This factor is related to the gas lifetime, so the longer lifetime the larger its GWP. In this sense, the comparative impact of the N_2O and CH_4 on climate change is over 298-fold (Forster et al., 2007; Myhre et al., 2013) and 25-fold (Myhre et al., 2013) greater than CO_2 , respectively, over a 100-year time horizon so CH_4 and N_2O are particularly

dangerous despite their lower atmospheric concentrations. In addition, N_2O is involved in the depletion of the ozone (O_3) layer, which shields the Earth from the sun's ultraviolet rays. When N_2O rises into the stratosphere, most of it is broken down into harmless molecules of N_2 and O_2 by the sun's rays. But some N_2O reacts with oxygen atoms to produce nitric oxide (NO), which is involved in the catalytic destruction of O_3 (Baird and Cann, 2012).

According to the IPCC, the agriculture, forestry and land-use sector (AFOLU) is responsible of almost a quarter (10–12 GtCO2eq/yr) of anthropogenic GHG emissions (IPCC, 2014). The causes of its contribution to the global budget are deforestation and losses from soils during nutrient cycling and livestock management. In terms of GHGs emitted, this sector can be a source (deforestation, peatland drainage) or a sink of CO_2 (reforestation, soil carbon sequestration) but it is thought to be a large source of CH_4 and N_2O, mainly caused by various agricultural activities (Figure 1.2). It was estimated that agriculture caused losses of non-CO_2 GHG between 5.2-5.8 $GtCO_2$ eq $year^{-1}$, about 10-12% of the global anthropogenic emissions in 2010 (FAOSTAT, 2013).

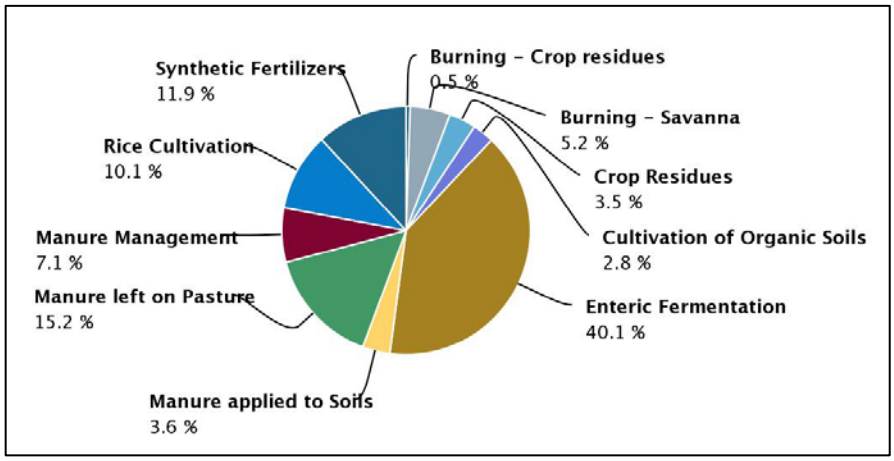

Figure 1.2. Average 1990-2011 distribution of the global non-CO_2 emissions (CH_4, N_2O) caused by agriculture. Source: FAOSTAT (2013).

Global anthropogenic non-CO_2 emissions from agriculture in 2011 had increased by 16.9% since 1990 (FAOSTAT, 2013). Data show that the main contributors to this increase were Africa, Asia and America, with average annual increases in the emission of these non-CO_2 gases for the period 1990-2011 of 1.79%, 1.53%, 0.91%, respectively. The opposite situation was observed in Europe where, over the same period a decrease of 2.13% was evidenced. This decrease in the European agriculture sector was mainly caused by the decline in livestock numbers, specially dairy cows which cause the largest annual GHG emissions in the EU- 27 (195 Mton CO2-eq) followed by the beef (192 Mton

CO2-eq) (Figure 1.3). The economic crises in Eastern Europe and the reduction in the use of artificial N fertilizer due to the implementation of the Nitrates Directive (91/676/EEC), regulation that protects water quality across Europe by preventing nitrates from agricultural sources and promotes the use of good farming practices, may have also contributed to the decreased emissions.

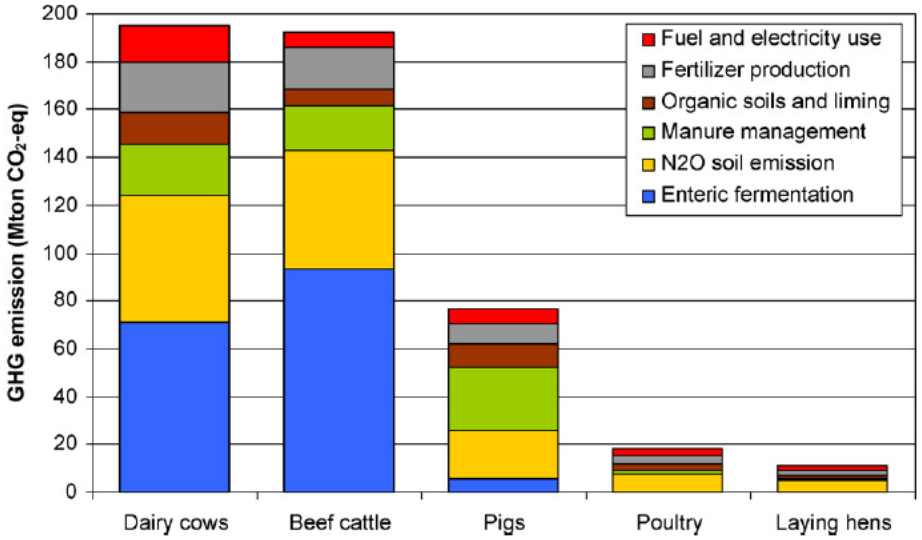

Figure 1.3. Total greenhouse emissions from the different emission sources associated with livestock production in the EU-27. Source: Lesschen et al.(2011a).

1.2. Nitrogen fluxes from agricultural soils

1.2.1. Nitrogen cycle in dairy farms

In farming systems, the natural nitrogen (N) cycle is modified with the purpose to produce food and fibre. This is possible by increasing the N inputs to soils and their mobility within plant soil/ecosystem. Annual N flows at within the farm are represented by the numbers shown in Figure 1.4. The main inputs of N are those from both imported animals or feed (1), animal bedding (2), mineral fertilizers and manures from outside the farm, seeds for obtaining crops, atmospheric deposition and N fixation by legumes (3). Nitrogen is exported from the farm as crops and straw (4), livestock sold or milk (5), manure (6) and there are losses of N_2 as well other the reactive forms of N to the atmosphere (ammonia (NH_3), N_2O and nitric oxide (NO)) (7,8,9) or run-off or leaching to water bodies (nitrate (NO_3^-), ammonium (NH_4^+), dissolved organic nitrogen (DON)) (10). The farm N cycle also involves internal transformations such as when the N no incorporated in animal protein or milk is excreted as dung and urine during grazing (11), or when N excreted during animal housing or animal holding areas (12) or is applied directly to land or after manure management (13,14,15). This added N

can be immobilized in organic N forms or even transformed by mineralization to forms available for plant uptake and/or for gaseous losses by NH_3 volatilization or N_2O emissions (19). These loses of the reactive N are increased compared to natural ecosystems, causing negative consequences for air and water quality, human health, biodiversity loss and climate change (Erisman et al., 2013; Fowler et al., 2013).

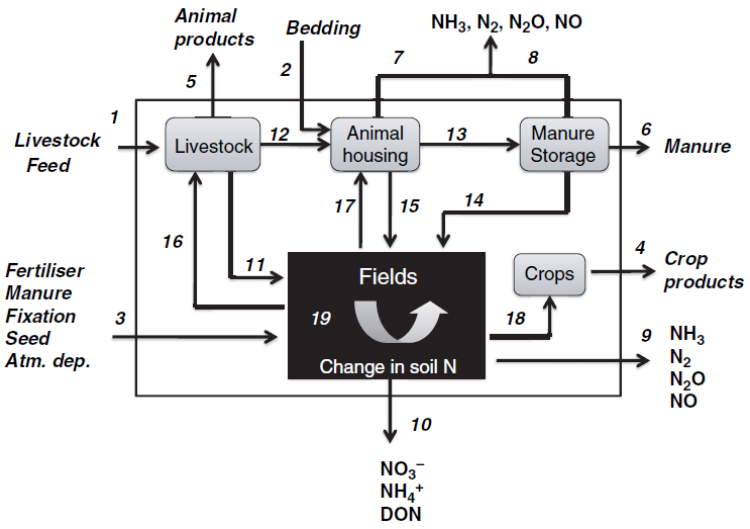

Figure 1.4. Schematic diagram of annual nitrogen flows on a farm. Atm. dep.= atmospheric deposition, DON = dissolved organic nitrogen. The numbers refer to the flow or transformation processes described in the text. Source: Jarvis et al. (2011).

1.2.2. Nitrous oxide production processes from soils

Agricultural soils are the main emitting sources of N_2O and it is produced mainly by a wide range of soil microorganisms (i.e. bacteria, fungi) which oxidize and reduce inorganic N forms existing in the soil. The inorganic N forms are used by these organisms as part of their electronic transport systems, for energy, as a source of electrons or as terminal electron acceptors. As a result of these reactions, different N gases are released to the atmosphere, including N_2O. Some of the reactions that cause N_2O production are represented in Figure 1.5.

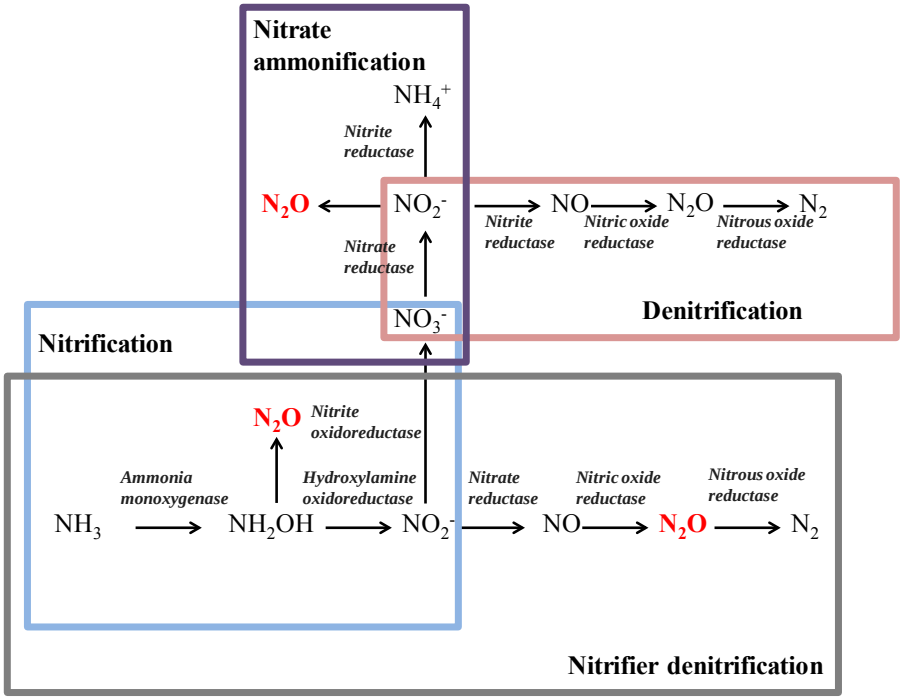

Figure 1.5. Biological sources of N_2O in soils. Adapted from Baggs (2008).

1.2.2.1. Nitrifier denitrification

Nitrifier denitrification is the pathway where ammonia oxidising bacteria can reduce nitrite (NO_2^-) to N_2 via N_2O during the ammonia oxidation process (Wrage et al., 2005). This transformation differs from nitrification in the fact that it does not produce NO_3^- (Figure 1.5) and also the process occurs under short-term O_2 limitation (Wrage et al., 2005). Most of the N_2O emitted from soils has been attributed to denitrification and, to a lesser extent to autotrophic nitrification (Bateman and Baggs, 2005; Saggar et al., 2009; Trogler, 1999; Williams et al., 1992). However, there are some studies which highlight the importance of nitrifier denitrification in the N_2O produced from soils (Kool et al., 2011; Venterea, 2007; Wrage et al., 2005). Wrage et al. (2005) studied the contribution of the different pathways of N_2O production in soils using a dual isotopic labelling method (single and double ^{15}N labelled ammonium nitrate, NH_4NO_3, with ^{18}O labelled water) and the conventional inhibitor methods with acetylene (C_2H_2) and O_2. Both isotopic and inhibitor methods identified nitrifier denitrification as the cause of 44 and 40% of the N_2O produced over 24 hours. Kool et al. (2011) using the same double-isotopic method obtained similar conclusions as Wrage at al. (2005) when moisture conditions are suboptimal for denitrification.

1.2.2.2. Nitrate ammonification

Another process that has recently been studied in soils is nitrate ammonification or dissimilatory NO_3^- reduction to NH_4^+ (DNRA), which occurs under even more anaerobic conditions than denitrification. The importance of DNRA is the fact that NO_3^- is transformed to NO_2^- and then to NH_4^+, which is less mobile and may conserve N in the ecosystem (Tiedje, 1988) (Figure 1.5). The main factor that influence the importance of this pathway are, in addition to the reducing (anoxic) soil conditions, high soil C/NO_3^- ratios. Large numbers of bacteria and fungi have the ability to perform DNRA. Bacteria involved in this process are either obligate anaerobes (*Clostridium*), facultative anaerobes (*Citrobacter, Enterobacter, Erwinia, Escherichia, Klebsiella*), aerobes (*Bacillus, Pseudomonas*) (Tiedje, 1988) and fungi of the genus Ascomycota (e.g. *Fusarium oxysporum*) (Zhou et al., 2002). Rütting et al.(2011) assessed the importance of DNRA in the terrestrial soil cycle based on published work where ^{15}N tracer techniques were used. They observed that the occurrence of DNRA is widespread and gross DNRA is reported from temperate and tropical forest soils, temperate grassland soils and even in an arable soil. According to a multiple linear regression developed in that study, the largest gross DNRA could be observed in humid regions but the importance of this process (in terms of % of NO_3^- consumed) can be larger in temperate climates.

1.2.2.3. Nitrification

Nitrification is an aerobic process performed by autotrophic and heterotrophic organisms. Autotrophic nitrifiers oxidize NH_4^+ and NO_2^- to obtain energy. In heterotrophic nitrification, bacteria use organic substances for C as energy source. In general, autotrophic nitrification is the dominant nitrification pathway in arable soils with heterotrophic nitrification being favoured in more acidic soils (Anderson et al., 1993; Bremner, 1997; Robertson and Groffman, 2007). In autotrophic nitrification (Figure 1.5), ammonium (NH_4^+) is oxidized to nitrate (NO_3^-) under aerobic conditions by bacteria. Nitrous oxide is formed during the first step of the nitrification process called ammonia oxidation where ammonia (NH_3) is oxidized to nitrite (NO_2^-) via hydroxilamine (NH_2OH) (Baggs, 2008; Baggs, 2011; Bremner, 1997; Wrage et al., 2005) by ammonium oxidizing bacteria. *Nitrosomonas* is the most frequently identified genus associated with this step, although other genera, including *Nitrosococcus,* and *Nitrosospira* and some subgenera of *Nitrosolobus* and *Nitrosovibrio* are also associated (Watson et al., 1981). In the second step of the process, the NO_2^- is oxidized to NO_3^- by genus *Nitrobacter*, *Nitrosospira* and *Nitrococcus.*

1.2.2.4. Denitrification

Denitrification is a microbial process which sequentially reduces NO_3^- to di-nitrogen (N_2) via NO_2^-, nitric oxide (NO) and N_2O utilizing organic carbon (C) as the electron donor under conditions with low soil O_2 concentrations (anaerobic) (Morley et al., 2014) (Figure 1.5). More than 60 genera of denitrifying microorganisms have been identified, which are within the bacteria, archaea, fungi and eukaryote kingdoms (Baggs, 2011). In terms of denitrifying bacteria, the most important genus are the heterotrophic facultative bacteria *Pseudomonas, Bacillus, Propionibacterium and Tiobacillus* (Wrage et al., 2001). Within the fungi, *Fusarium* and other fungi such as *Ascomycota* (e.g. *Cylindrocarpon tonkinense*, *Gibberella fujikuroii*), and *Basidiomycota* (e.g. *Trichosporon cutaneum*) are important (Shoun et al., 1992; Shoun and Tanimoto, 1991).

1.2.3. Factors influencing N_2O production

Although N_2O can be produced by several pathways in soils, nitrification and denitrification processes from managed and natural soils are responsible of approximately 56-70% of the global N_2O emissions (Braker and Conrad, 2011; Syakila and Kroeze, 2011). The factors that affect N_2O production have been described as proximal or distal (Robertson, 1989). Proximal factors are those that immediately affect microbial communities resulting in instantaneous changes in N_2O rates and distal factors are those that control the composition and diversity of denitrifying communities over larger spatial and temporal scales for a longer term and on a larger scale than proximal regulators.

1.2.3.1. Proximal factors

1.2.3.1.1. Soil moisture and aeration

Soil moisture is a major factor affecting N_2O production as it regulates the oxygen availability in the soil for microbial activity. In wet soils the proportion of air-filled pores is low so O_2 diffuses much slower (Renault and Stengel, 1994). In a large number of studies (e.g. Bateman and Baggs, (2005), Davison, (1991), Dobbie and Smith (2003), Weier et al. (1993)) soil moisture is expressed as % water filled pore space (WFPS) which denotes the proportion of the total porosity occupied by water and therefore the aerobic and anaerobic conditions in a soil. Nitrification is observed in the WFPS range of 30-70% and denitrification becomes the dominant process at WFPS values above 60% (Figure 1.6). However, when WFPS exceeds 80%, N_2O production by denitrification decreases probably because of

the rapid initialization of strictly anaerobic conditions, resulting in the formation of N_2 rather than N_2O (Figure 1.6).

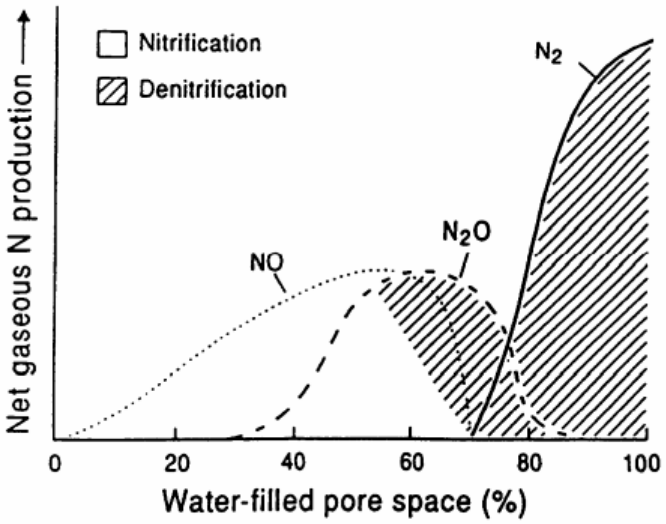

Figure 1.6. Relationship between soil WFPS and N_2O, NO and N_2 fluxes. Source: Davidson (1991).

1.2.3.1.2. Temperature

According to many studies (Singh et al., 1993; Stark, 1996; Stark and Firestone, 1996), the optimum temperature for nitrification is environment specific. Most of ammonia oxidizing bacteria have an optimum growth in the range of 25-30°C. However there is evidence that nitrifier activity can happen under colder temperatures in winter (2-10°C) (Avrahami and Conrad, 2005; Cookson et al., 2002). Denitrification is very sensitive to high temperatures as it enhances microbial respiration, resulting in the depletion of the O_2 existing in the soil (Smith et al., 2003). Normally the dependence of temperature is expressed in terms of 'Q_{10}', which is the rate of change of the microbial process when temperature is increased by 10°C. Dobbie and Smith (2001) obtained Q_{10} values of 50 for the 5–12°C and 8.9 for 12–18°C intervals in an arable soil meanwhile the corresponding Q_{10} values for the grassland soil were 3.7 and 2.3.

1.2.3.1.3. Available carbon and nitrogen

Mineral N (NH_4^+, NO_3^-) forms are required for the development of the nitrification and denitrification processes so increasing the availability of these N forms in the soil by applying N fertilizers (mineral fertilizers or manures) or even urine and dung during grazing may increase N_2O emission rates from

both pathways. Available C influences nitrification and denitrification reactions (Bremner 1997), because it can stimulate microbial growth and activity, and is also the electron donor required for denitrifiers (Cameron et al. 2013). Additionally, available C also influences in the oxygen status in aerobic soils as it increases heterotrophic respiration in aerated soils and thus O_2 consumption, generating the anaerobic conditions necessary for denitrification (Groffman et al., 1988).

1.2.3.1.4. pH

Soil pH is also an important variable that affects the N_2O produced by denitrification and nitrification processes. When denitrification is the main pathway of N_2O emission, higher pH values decrease the soil N_2O emissions, but if nitrification is the main process of N_2O production, then an increase in the soil pH stimulates N_2O production. Van Den Heuvel et al. (2011), in a laboratory experiment, tested the effects of a range of pH between 4 and 7 on soil NO_3^- reduction, N_2O and N_2 production rates. They observed that the N_2O:N_2 emission ratio decreases with pH because the enzyme nitrous oxide reductase is inhibited by low pH. Similar conclusions were obtained by Čuhel et al.(2010) after an *in situ* experiment where they added ^{15}N-labeled NO_3^- to acidic, neutral pH, and alkaline soils. According to Šimek and Cooper,(2002), there is no optimum pH for denitrification as denitrifies can adapt to soil pH but for nitrification Kyveryga et al.(2004) reported an optimum pH of almost 8 (>7.5).

1.2.3.2. Distal factors

1.2.3.2.1. Soil texture and composition

Nitrous oxide emitted is also influenced by soil texture, which influences water movement in the soil and consequently, soil WFPS. In general, fine textured soils with poor drainage conditions are likely to emit more N_2O for longer periods than coarse soils with well-drained conditions (Barton et al., 1999; Bouwman et al., 2002; Clayton et al., 1997; Saggar et al., 2004a). However, when these fine textured soils have very poor-drainage, as in Burchill et al. (2014), high rainfall results in low N_2O emissions because of completely saturated conditions, which are favourable for complete denitrification (N_2 production).

1.2.3.2.2. Weather conditions

The pattern of precipitation and temperature has direct effects on soil functioning as they are key in the temporal changes in soil moisture contents and temperature, and thus, N_2O emissions. Both parameters are highly specific and vary between soils and climates. In addition to their biochemical effects on N_2O emissions (Section 1.2.2), they also have physical effects such as freeze-thaw and dry-wetting cycles and also biological effects that result in changes in microbial populations. Freezing and thawing caused by low temperatures are of special interest as they lead to emissions with significant or dominant contributions to the annual N_2O budget according to observations by Burchill et al. (2014), Lampe et al. (2006) and Syväsalo et al.(2004) in grassland soils and by Flessa et al. (1995) in arable soils. These N_2O emissions are caused by the release of C and N from soil aggregates that rupture or microbial cells that lyse during the freezing periods. The released C and N are then available for denitrifiers during thawing. Additionally, the low temperatures may suppress N_2O reductase activity and therefore alter the N_2O/N_2 emission ratio (Melin and Nômmik, 1983; Müller et al., 2002; van Bochove et al., 2000). Likewise, drying-wetting cycles also cause large losses of N_2O as it has been reported in studies in temperate climates (Jørgensen et al., 1998; Kim et al., 2010; Louro et al., 2015), semi-arid climates (Barton et al., 2008) and under laboratory incubations (Bergstermann et al., 2011). Some reasons to explain those N_2O emissions caused by these cycles are: 1) reduction in the microbial population and therefore increased levels of microbially derived organic matter (OM) in the soil during the dry period which the surviving microbial population mineralizes after soil rewetting (Van Gestel et al., 1993) or 2) disruption of soil aggregates during drying period and exposition of soil OM to a rapid mineralization (Goebel et al., 2005).

1.2.3.2.3. Soil organic matter: mineralization-immobilization

Nitrogen mineralization is the process by which microbes convert organic N to plant-available inorganic forms, whereas immobilization is the process where microorganisms take-up inorganic N forms and convert them into organic forms. The ratio C/N of the OM controls the balance of two soil biological processes, immobilization-mineralization. Low C/N ratio (generally less than 30 parts of C:1 part of N) means that the soil organic matter, or that incorporated with residues, supplies enough N to meet the needs of decomposing organisms so mineral N is released through mineralization and is available for plant uptake or N_2O losses. Contrary, when OM has a high C/N, the supplied N is not enough so microorganisms require more N (as NH_4^+ or NO_3^-) to decompose the C from the organic materials and N is immobilized until these microorganisms die and N is released. Crop residues

incorporated in the soil are a potentially important source of N_2O as: i) they provide a supply of easily mineralizable N, which may be transformed into mineral N, ii) they provide a supply of easily mineralizable C, which may enhance denitrifier activity and therefore N_2O emissions from both soil mineral N and crop residue N, and iii) they may cause a local increase of the oxygen consumption in the soil leading to anaerobic soil conditions ideal for denitrification (Lesschen et al., 2011b). Baggs et al. (2000) observed that the large N_2O emissions after incorporation of lettuce residues (C:N ratio: 7.5:1) were probably caused by the stimulation of mineralization, contrary to winter wheat straw incorporation (C:N: 38:1) which probably caused temporal immobilization.

1.2.3.2.4. Animal excreta

A large fraction of the anthropogenic N_2O emissions come from grazed grasslands. The reason is that little of the N ingested by livestock from feed is used efficiently with 75-95% being excreted as urine and faeces (Eckard et al., 2010; Saggar et al., 2013). Dairy cows excrete between 197 to 534 g N per day (Aguerre et al., 2011; Bossuet et al., 2006; Burke et al., 2008; Spek et al., 2013). In urine (68-75% of excreted N), livestock excretes N mainly as urea (64-94% of urine N) but also other compounds as allantoin, hippuric acid, creatinine, ammonia, creatine, free amino acids, uric acid, xanthine and hypoxanthine (Bussink and Oenema, 1998). In dung, the majority of N is organic N. While grazing, excreted N is deposited unevenly in localised areas where livestock deposit between 800-1300 kg N ha^{-1} in a single cow urination patch (area of 0.16 to 0.49 m^2) and 2000 kg N ha^{-1} in a single dung patch (area 0.05 to 0.09 m^2) (Eckard et al., 2010; Haynes and Williams, 1993; Lantinga et al., 1987). These application rates are more than the soil-plant system can efficiently use so N_2O production and emission is very high especially when grazing and N fertilization are combined (up to 51.3 kg N_2O-N ha^{-1} $year^{-1}$, Rees et al. (2013)). Also, intense animal treading compacts soil, increasing soil WFPS and thus, causing more anaerobic microsites in the soil that are optimal for denitrification (Drewry et al., 2008; Simek et al., 2006). De Klein *et al.* (2006) observed that restricting grazing patterns to 3h per day during the wettest period of year (autumn/winter) reduced N_2O emissions by 40% and prevented sward damage. Similar results were observed by Luo *et al.* (2008) who obtained reductions of 60% in the total N_2O emissions during autumn-winter when livestock grazed 6 h instead 24 h.

1.2.3.2.5. Fertilization

Mineral fertilizers and manures are generally used in dairy farms to increase crop yields but they also cause large N_2O emissions. Manures are valuable fertilizers as they increase productivity of the land and nutrient cycling as they provide nutrients (N, P, K) for plant nutrition, enhance crop growth and improve soil structure which is important for air and water circulation, water retention and for greater penetration of crop roots. They also improve biodiversity as they contain OM which provides nutrients and energy for microorganisms. The literature reports different losses of N_2O after organic and mineral fertilizer applications as the different studies are influenced by soil and environmental conditions. Velthof and Mosquera (2011) observed higher N_2O emissions from soils fertilized with calcium ammonium nitrate (CAN) than with slurries in grasslands and the opposite result in maize cropped soil. Jones et al. (2007) reported losses of N_2O of 1.4% of the N applied after application of ammonium nitrate (NH_4NO_3) and 0.5% after cattle slurry spreading during the first year of measurements but no significant difference in the second year. Similar results were also observed by Bourdin et al. (2014) who reported losses of 1.6% and 0.8% of the N applied as CAN and slurry, respectively. However, manure applications may affect N_2O emissions as they contain large quantities of labile C (contrary to mineral fertilizers) which may increase microbial respiration rates, thus depleting oxygen and providing anaerobic conditions for denitrification. Pelster et al. (2012), in a wheat cropped soil, and Chantigny et al. (2010), in a maize cropped soil, observed that the larger N_2O emissions from manure applications compared to mineral fertilizers were related to low soil C contents, which is commonly observed in coarse-textured soils.

Slurry can be applied to soil by using techniques such as surface broadcast, band spreading, trailing shoe or injection into soil (Figure 1.7). Slurry injection is commonly used to avoid losses of N by NH_3 volatilization (see Section 1.2.4.1), however, some studies have reported larger denitrification rates (N_2O+N_2) after slurry injection respect to other techniques. Dosch and Gutser (1996), using the acetylene technique, observed denitrification losses of 7.5 kg N ha^{-1} from injected slurry compared to 4.5 kg N ha^{-1} after surface band slurry application 100 days after slurry applications. Wulf et al. (2002) also observed larger N_2O emissions after slurry injection to arable and grassland soils compared to application by splash plate, trail hose and incorporation. Velthof and Mosquera (2011) also observed that 0.9% and 0.4% of the N applied was lost when cattle slurries were injected and surface-applied, respectively, to maize soils and losses of 0.4% and 0.1% of the N applied when similar techniques were used in a grassland soil. They also observed similar results when pig slurry was used in the same maize soil, causing even higher losses than cattle slurry (3.6 and 0.9% of the N applied by injection and surface application, respectively). However, other studies as Velthof et al. (1997) and Vallejo et

al. (2005) did not find a significant difference between manure application techniques in grassland on total denitrification losses.

As it was observed from the experiments cited above, site specific conditions, weather, crop, type of fertilizer and application technique influence losses of N_2O and these losses are sometimes overestimated when using the default value of 1% proposed by the Intergovernmental Panel on Climate Change (IPCC) (IPCC, 2006). Lesschen et al. (2011b), using studies that compared N_2O emissions for different N inputs and environmental factors, developed a inference scheme to quantify European N_2O EFs based on the INTEGRATOR model. They compared the calculated N_2O emissions from agricultural soils with the emissions calculated using the default IPCC EF and observed that there are clear regionally differences between the EF inference scheme and the IPCC EF (Figure 1.8). They observed that on the Western part of UK and Ireland and the Northern of Spain, N_2O emissions from soils are higher due to the large annual precipitation. They also observed the effect of soil type in the Netherlands. According to the EF inference scheme, the largest N_2O emissions occur in Western parts where soils are mainly clay and peat, while the Eastern and Southern parts have lower N_2O emissions because soils are mainly sandy.

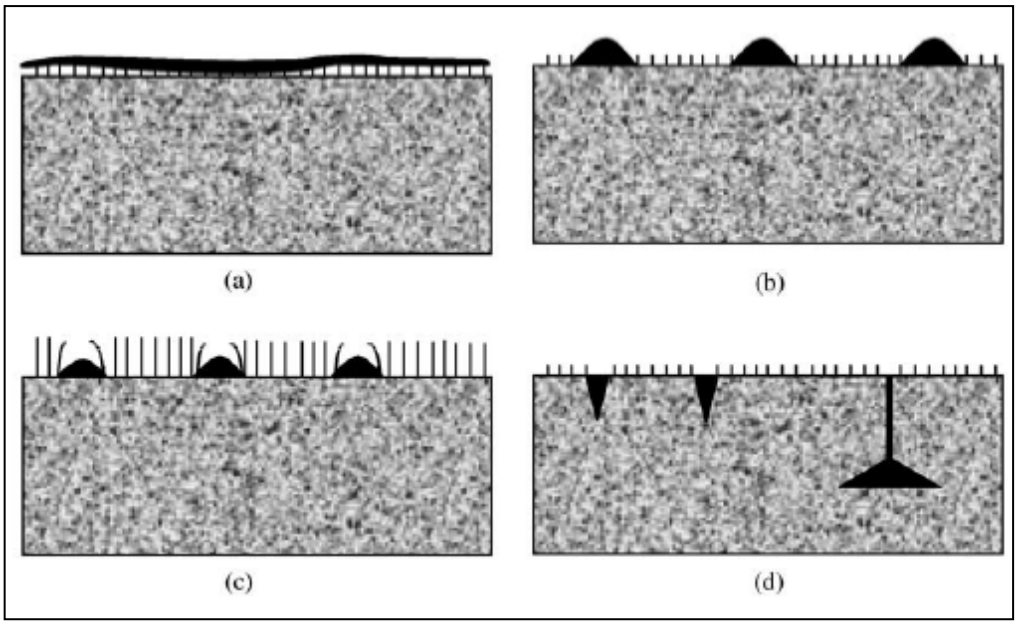

Figure 1.7.Schematic representation of slurry applied to soil using different application techniques. a) surface broadcast-uniform covering of slurry across the crop; b) band spreading-slurry placed in discrete bands on the crop by trailing hoses; c) trailing shoe-slurry placed in discrete bands on the soil surface below the crop canopy; d) injection-slurry placed in shallow, open slots or deep, closed slots within the soil. Source: Misselbrook et al. (2002).

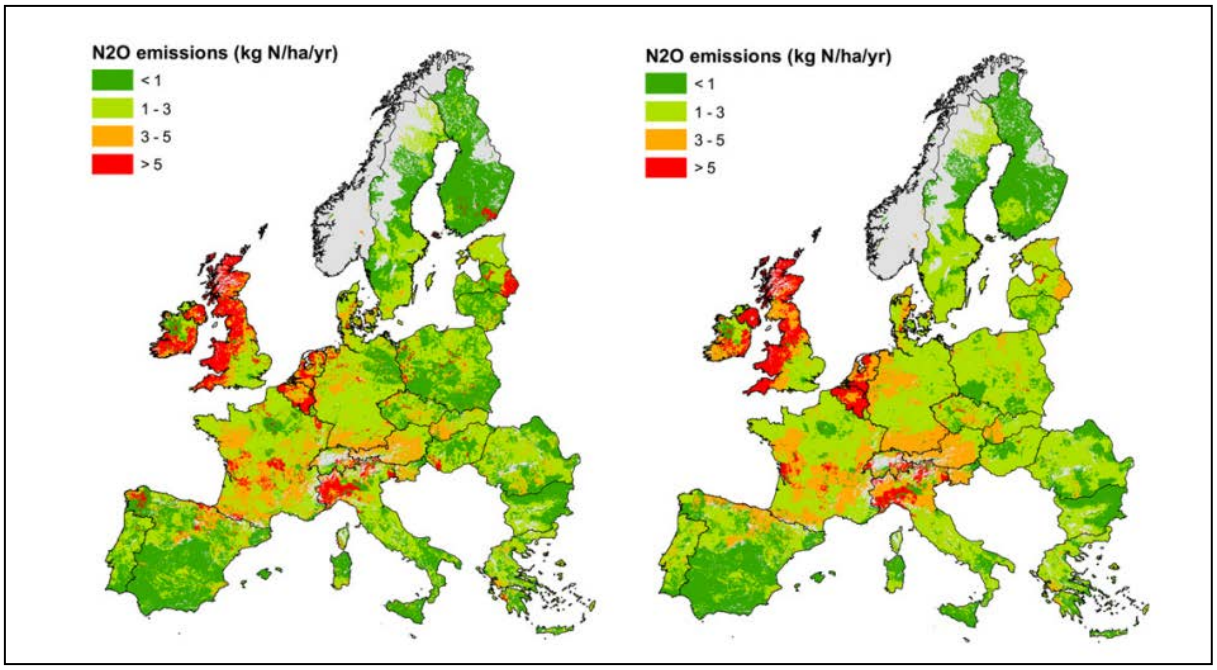

Figure 1.8. Comparison of calculated N_2O soil emissions based on the N_2O EF inference scheme (left) and the default IPCC EF of 1%. Source: Lesschen et al.(2011b).

1.2.3.2.6. Nitrogen fixation

Nitrogen is an essential nutrient for plant growth as it is required for the synthesis of biomolecules (i.e. amino acids, proteins, nucleic acids). Nitrogen is stored in soil as OM so this element is often limiting for most plants and animals as, generally speaking, they can only take up N when it is as the inorganic forms of ammonium (NH_4^+-N) and nitrate (NO_3^--N). So, in ecosystems where N fertilizers are not applied, soil N comes mainly from the atmosphere, which is the largest N pool and contains almost 78% of the total N (N gas) available on Earth. Most plants and animals cannot use N directly as gas as it is in a stable form because of the triple bond that holds the two atoms of N (Galloway et al., 2004). Instead they rely on N being fixed, that is pulled from the air and bonded to hydrogen and oxygen to form inorganic compounds they can use. Nitrogen fixation is carried out by a number of fixing organisms that live in soils but the most important ones are those that form symbiotic relationships with higher plants, such as rhizobia, which can be found in nodules of the roots of legumes. Symbiotic nitrogen fixation by rhizobia in legume root nodules injects approximately between 40 and 100 million tonnes of N into agricultural systems each year (Udvardi and Poole, 2013; Vitousek et al., 2013). These bacteria have enzymes that can convert N gas directly into N reactive forms which include ammonia (NH_3), ammonium (NH_4^+), nitrogen oxides (NO_x), nitric acid (HNO_3), nitrous acid (HONO),

other organic N compounds (such as urea, nucleic acids, amino acids, proteins) and also nitrous oxide (N_2O) (Fowler et al., 2013).

1.2.3.2.7. Plant N uptake

The primary consideration for mitigating N_2O emissions from agricultural lands is to match the supply of mineral N (from fertiliser applications, legume-fixed N, organic matter, or manures) to the spatial and temporal needs of crops/pastures/trees as N loaded in excess is susceptible to produce direct or indirect losses of N_2O. An increasing number of studies report greenhouse gas emissions as a function of crop yield rather than as a function of land area, based on the work by Mosier et al. (2006) and van Groenigen et al. (2010) . Mosier et al. (2006) proposed the concept of greenhouse gas intensity which relates to GWP resulting from N_2O, CH_4 and CO_2 emissions to crop yield (grain yield for maize). In adittion, van Groenigen et al. (2010) introduced the concept of 'yield-scaled N_2O emissions' which relates the N_2O emitted from cropping systems with the resulting yield. In the latter study, a meta-analysis was performed based on a review of the literature on non-legume annual crops (maize, wheat, potato, onion and flooded rice) to relate N application rates, N uptake (above-ground N uptake), nitrogen use efficiency (NUE), and N_2O emissions (Figure 1.9). They postulated that in a world with increasing food demand and limited land area for expansion of agriculture, agronomic conditions should be included when assessing N_2O emissions and that requires the expression of N_2O losses as function of crop yield. They observed that yield-scaled N_2O emissions were lowest where crops were grown close to their yield potential with high N use efficiency and the increase in yield-scaled emissions only occurred at high or excessive N rates. They observed a negative and significant relationship between yield-scale N_2O emissions and NUE. They observed decreases from 12.7 to 7.1 g N_2O-N kg^{-1} N uptake when NUE increased from 19 to 75% (Figure 1.9).

1.2.4. Indirect N_2O emissions from soils

1.2.4.1. Ammonia volatilization

Ammonia volatilization is the gaseous loss of NH_3 after conversion from NH_4^+. This process is important as NH_3 loss is a direct loss of plant available N and also causes acidification and eutrophication of natural ecosystems as it returns to the Earth's surface through wet deposition (dissolved in rainwater) or dry deposition (attached to particulate matter) (Cameron et al., 2013). In addition, NH_3 re-deposition represents an indirect source of N_2O. Bussink and Oenema (1998) reviewed NH_3 losses on dairy farms, which mainly occur during manure applications but also during

housing, manure storage, grazing, urea-based fertilizer applications and crops. In terms of manure application, NH_3 volatilization is especially important during the first 24h after land application and the quantity of NH_3 lost depends on factors such as characteristics of the material applied (% dry matter (DM), total N, pH, NH_4^+), environmental factors (temperature, wind and rainfall) and application technique (Bourdin et al., 2014; Cameron et al., 2013; Dell et al., 2011; Génermont and Cellier, 1997; Huijsmans et al., 2003; Misselbrook et al., 2005; Misselbrook et al., 2004a; Smith et al., 2000; Søgaard et al., 2002; Sommer and Olesen, 1991). Briefly, according to these studies, NH_3 volatilization increases with increasing %DM content of the manure applied. This is due to the fact that manures with large amount of solids tend to have lower fluidity and, therefore less infiltration into the soil, leaving NH_4^+ exposed to volatilization. Ammonia volatilization increases when manure temperature increases, especially within the first hours after manure application. This is also enhanced with the absence of rainfall and under windy conditions during manure application. Absence of rainfall reduces NH_4^+ infiltration into the soil therefore increasing its exposition to air and wind and in turn enhances the mass transfer and gas exchange between the manure and atmosphere. In terms of application technique, injection is the technique that causes less NH_3 emissions from manure applications as decreases the exposition of NH_4^+ to air, resulting in reductions in the NH_3 volatilized by between 40 to 90% with respect to surface applications.

Søgaard et al. (2002), based on empirical data of NH_3 loss in experiments from seven European countries after land applications of cattle and pig slurries, developed a multiple regression model (ALFAM) that uses a Michaelis-Menten type equation to predict NH_3 loss over time (t). This regression model (R^2=80) provides the estimated losses as response to variables that significantly affect NH_3 volatilization such as soil water content, air temperature, wind speed, slurry type, dry matter content of slurry, total ammoniacal nitrogen content of slurry (TAN), application method and rate, slurry incorporation and measurement technique. Bourdin et al.(2014) validated the NH_3 emissions data obtained after application of cattle slurries by mimicking trailing shoe and splash plate with ALFAM estimations and obtained a good correlation (R^2 0.94).

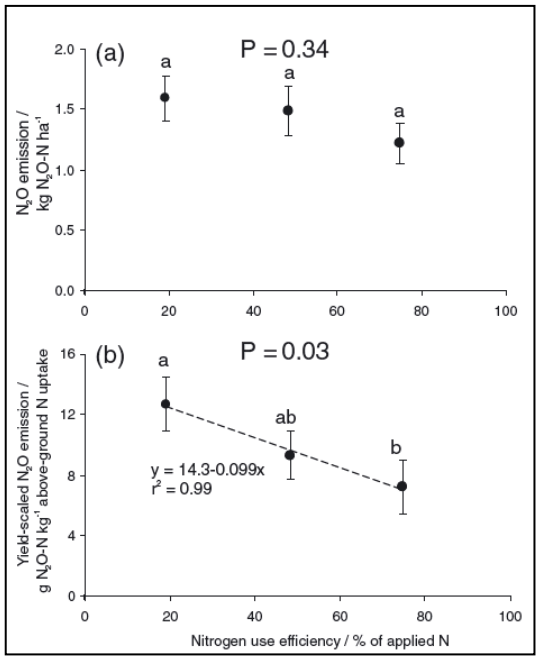

Figure 1.9. Meta-analysis results of the relationship between nitrogen use efficiency (NUE, expressed as apparent recovery efficiency (in %) of N applied in kg N taken up by plants per kg of N) and yield-scaled N_2O emissions. Nitrogen use efficiency plotted against a) N_2O emission per ha, and b) N_2O emission per kg of above-ground N uptake. The P value denotes the overall significance of difference between classes. Classes with different numbers are significantly different, based on a post-hoc analysis with α=0.05. Error bars denote standard errors (n=14). The hatched line in b) denotes a fitted regression model. Source: van Groenigen et al. (2010).

1.2.4.2. Nitrogen losses to groundwater

Nitrogen lost into aquatic systems via leaching is also susceptible to be denitrified and result in N_2O emissions. The size of the N pool susceptible to be leached may be determined by the soil N balance between N inputs from synthetic and organic fertilizers, urine, dung deposition, SOM, mineralization, atmospheric deposition and N removal, and N soil cycling by immobilization into SOM, denitrification, NH_3 volatilization and plant uptake (Jarvis et al., 1995). Estimates of N leached from managed grasslands vary widely, ranging from about 5 to 200 kg ha^{-1} $year^{-1}$ and this is due to many factors including differences in N input, N output in excreta, soil drainage and animal type (Monaghan et al., 2007). Most of the leached N from grazed and fertilized grasslands come from the urine-N deposited by livestock as this load is above plant N uptake and the excess of nitrate remains in the soil until drainage and leaching occurs. Di and Cameron (2002) and Silva et al. (1999) reported losses of up to 124 kg N ha^{-1} $year^{-1}$ from urine patches, which contrasted with low losses (<8 kg N ha^{-1} $year^{-1}$) from the inter-urine patch areas. In grasslands for silage cutting, losses of N are lower. Estavillo et al.

(1995) observed leaching losses in the range 1.6- 5.1% of the N applied (120- 290 kg N ha^{-1} $year^{-1}$ as slurry and CAN) from a poor drained clay grassland soil in the Basque Country. They also reported that these losses were not as important as denitrification losses. In New Zealand, Di et al. (1998) reported N leaching losses between 2.5 and 9.8% of the N applied (400 kg N ha^{-1} $year^{-1}$) with a dairy effluent or mineral fertilizer leaching to a sandy loam soil. This shows that nitrate leaching is generally greater from sandy soils than clay soils because of the slower water movement and the greater potential for denitrification to occur in clay soils, which itself poses a greater risk of gaseous emissions. Temporally, the largest risk of N leaching is usually during late autumn, winter or early spring months where plant N requirements are low and rainfall inputs are high. In arable soils, autumn rainfall can also leach residual fertiliser nitrate left after the crop has been harvested and nitrate released by OM mineralisation. A dry summer can result in an accumulation of nitrate in the surface soil (because of low plant growth and low N uptake) and this nitrate can be leached over the subsequent winter. Cameron et al. (2013) reported annual losses from maize cropped soils of between 11 and 107 kg N ha^{-1} with much of this loss being attributed to the period between November and May before the crop is grown. They also argued that most of NO_3^- leached often comes from the mineralization of the organic-N and re-mineralization of fertilized-N that was immobilized earlier during previous cropping phase.

1.3. Methane fluxes from soils

In European agriculture dairy farms, animal enteric fermentation and manure management are direct sources of CH_4. In soils, CH_4 fluxes are the net result of CH_4 production by methanogenesis (+) and CH_4 oxidation (-) by methanotrophy processes (Baggs et al., 2006; Ball et al., 1999).

1.3.1. Methane production or methanogenesis

1.3.1.1. Mechanisms

Methanogenesis is a microbial process that may occur in soils when OM is degraded under anaerobic conditions and produces CH_4 and CO_2, under low concentrations of sulphate and nitrate. Methanogenesis can take place in anaerobic soils, such as rice paddies and wetlands, that are rich in organic matter, which are significant methane sources. This process (Figure 1.10) requires successive reactions which degrade molecules from complex to simpler compounds (Le Mer and Rogert, 2001; McCarty, 1982):

- Hydrolysis of biological polymers into monomers such as glucides, fatty acids, amino acids. This step is performed by hydrolytic microflora that are either aerobic, facultative or strictly anaerobic.

- Acidogenesis from the monomers and intermediary compounds formed during fermentation to produce volatile fatty acids, organic acids, alcohols, hydrogen (H_2) and CO_2. This step is performed by fermentative miroflora that can be either facultative or strictly anaerobic.
- Acetogenesis from the previous metabolites to form H_2, CO_2 and acetate by syntrophic or homoacetogenic microflora.

- Methanogenesis where the metabolites obtained during the acetogenesis step are used by methanogenic microflora to produce CH_4. In most environments where OM decomposition is important (digesters, freshwater, sediments and submerged soils), the main pathways are acetoclastic (cleavage of acetate) and hydrogenotrophic methanogenesis (CO_2 reduction by H_2) (Conrad, 2005). These bacteria are members of the domain Archaea, or archaebacteria (Whitman et al., 2006) and although most of methanogenic species cannot use acetate as C and energy source, acetotrophy methanogenesis is considered the cause of the bulk of CH_4 produced.

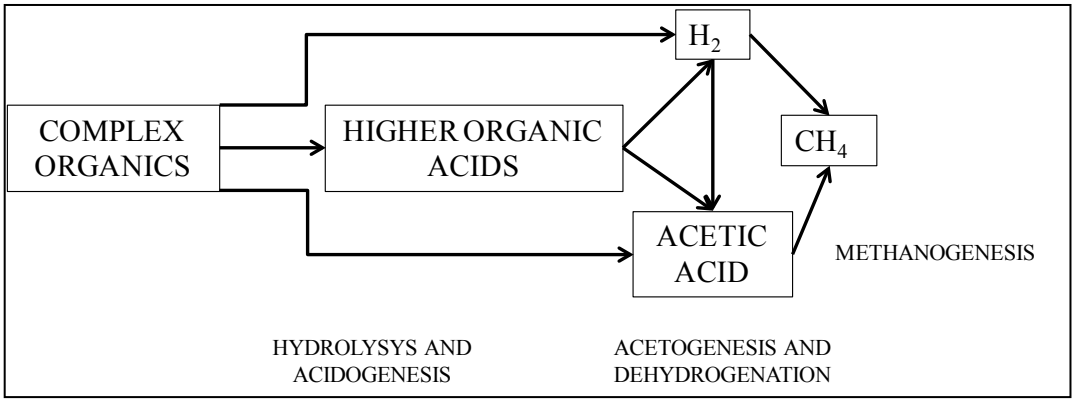

Figure 1.10. Stages of methane fermentation. Adapted from McCarty (1982).

1.3.1.2. Factors influencing the rate of CH_4 production

1.3.1.2.1. Soil aeration

In soils, methanogenesis occurs in environments such as wetlands and rice fields where conditions are highly reduced (Bachoon and Jones, 1992) (Eh<-200 mV, Le Mer and Rogert (2001)). The reason of the low oxide-reduction conditions is due to fact that methanogens are strict anaerobes and the presence of oxygen leads to the formation of reactive oxygen species, which damage cell membranes, DNA and proteins and, in particular, promote the dissociation of the crucial electron transporter (F420-hydrogenase enzyme complex) in methanogenesis (Schönheit et al., 1981; Storz et al., 1990).

1.3.1.2.2. Presence of inorganic redox substances

Methanogens are poor competitors for hydrogen and acetate in the presence of substances such as NO_3^-, iron (Fe^{3+}), sulphates (SO_4^{2-}) and manganese (Mn^{4+}) (Le Mer and Rogert, 2001; Whitman et al., 2006). Thus, even in the absence of O_2, hydrogenotrophic and acetolastic methanogenesis only takes place when these substances are depleted (Kumaraswamy et al., 2001; Segers and Kengen, 1998; Zehnder and Stumm, 1988). Kumaraswamy et al. (2001) tested the effects of the adittion of these substances on CH_4 production in anoxic rice soil samples. They observed that SO_4^{2-} was the most inhibitory compound for methane production followed by Fe^{3+}, NO^{3-}, and Mn^{4+}.

1.3.1.2.3. Available organic matter

Once anaerobiosis is established, organic matter is considered as the major limiting factor for methane production. Both the addition of direct methanogenic substrates like hydrogen or acetate, and the addition of indirect substrates like glucose and leaf leachate, enhances methane production in anaerobically incubated soil samples (Amaral and Knowles, 1994; Bachoon and Jones, 1992; Valentine et al., 1994).

1.3.1.2.4. Activity of methanogenic population

Limitation of methane production by the microbial biomass occurs when microbial uptake capacity does not meet by substrate supply. In principle, it can be a result of a periodical damage to methanogenic population due to poisoning as result of aerobiosis or starvation, due to competition for substrates with aerobic microorganisms (Segers and Kengen, 1998).

1.3.1.2.5. Temperature

Methanogens are very responsive to temperature changes within their functional temperature range. Most methanogens are able to function in a temperature range of about 20–40°C. Laboratory incubations of peat soil showed Q_{10} values of 5.6-16 for temperatures between 0 and 35°C (Dunfield et al., 1993).

1.3.1.2.6. pH

The pH range for methanotrophic activity in soils is wide. Most methanogens grow in the pH range of 6.0–8.0 but some can function under acidic conditions (pH< 3.5), as is the case for the genera *Methylocella and Methylocapsa* and species of *Methylocystis* isolated from peatlands and acidic forests (Dedysh et al., 2007; Dedysh et al., 2002).

1.3.2. Methane consumption or methanotrophy

1.3.2.1. Mechanism

Under aerobic conditions, soil methanotrophs communities can oxidize the CH_4 produced in anaerobic parts of the soil and atmospheric CH_4 (Figure 1.11). There are two distinct populations which have been found in most of soils: low capacity oxidizers (also 'high affinity oxidation') which are those that receive CH_4 only by diffusion from the atmosphere (e.g. forest soils) and those adapted to oxidize high CH_4 concentrations in the soil ('low affinity oxidation') (e.g. in wetlands, landfill and peat soils) (Le Mer and Rogert, 2001; Reay and Nedwell, 2004). Methane is oxidized to carbon dioxide by sequential reactions catalyzed by the enzymes methane monooxygenase (Methane MO), methanol dehydrogenase (Methanol DH), formaldehyde dehydrogenase (Formaldehyde DH), and formate dehydrogenase (Formate DH). Methane monooxygenase also catalyzes the oxidation of ammonia to hydroxylamine, which is then further oxidized to nitrite with the accompanying production of nitrous oxide. The cofactors nicotinamide adenine dinucleotide (NADH) and pyrrolo-quinoline quinone (PQQ) participate as electron carriers (Topp and Pattey, 1997).

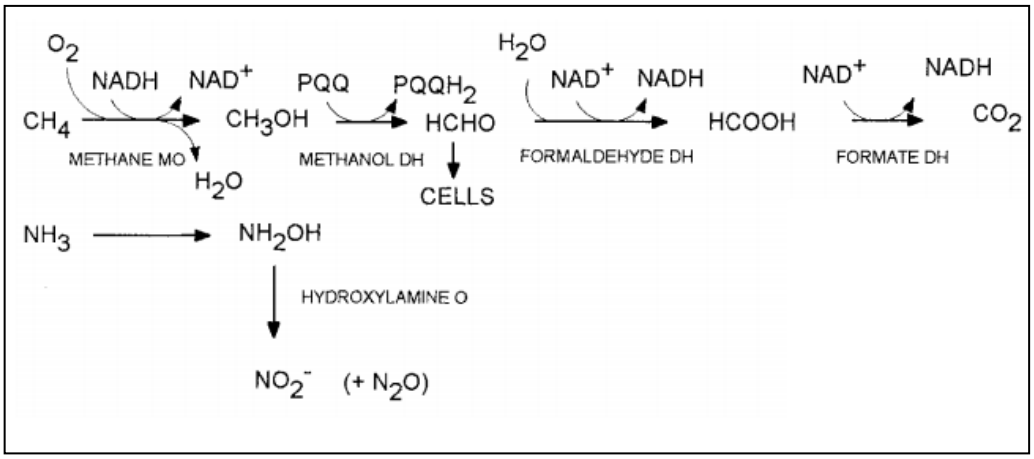

Figure 1.11.The pathway of methane oxidation common to all methanotrophs isolated to date. Source: Topp and Pattey (1997).

1.3.2.2. Factors influencing the rate of CH_4 oxidation

1.3.2.2.1. Soil aeration, temperature and pH

Methanotrophs derive energy from the oxidation pathway and utilize the intermediate formaldehyde as their sole or primary C source for growth. Oxygen is required as a terminal electron acceptor and is also an obligate reagent in the first reaction of the methane oxidation pathway catalyzed by the enzyme Methane MO. This requirement for oxygen to "prime" the methane oxidation pathway explains why methane is not consumed under highly reduced soil conditions. In terms of pH, methanotrophs have similar behaviour as methanogenic bacteria. They are generally mesophilic and relatively unresponsive to temperature changes. Dunfield et al.(1993) reported from laboratory incubations Q_{10} values of 1.4-2.1 in the temperature range of 0-35°C. Temporal patterns of CH_4 uptake have been observed in many studies. The resulting fluxes follow the general patterns of soil moisture content (expressed either gravimetrically or as WFPS) and also temperature (Mosier and Delgado, 1997; Saggar et al., 2007). It is observed that methane consumption increases during the dry periods due to the effect of soil water on the effective diffusivity of the soil. For example, Saggar et al. (2007) observed in grazed and ungrazed soils mean daily CH_4 flux of -2.22 g CH_4-C ha^{-1} day^{-1} during summer months, three times the consumption during the wet winter (−0.68 g CH_4-C ha^{-1} day^{-1}) where soils emitted more CH_4 because of soil saturated conditions after heavy rain (Figure 1.12). Mosier and Delgado (1997) observed in three types of grassland soils that CH_4 uptake rates were highest in February-April (lowest soil water content) and lowest in November (highest water soil contents).

Dobbie and Smith (1996), in a soil planted with wheat, observed a relationship between CH_4 oxidation rate and soil moisture content during the dry conditions observed in summer and in a woodland soil with soil moisture and temperature.

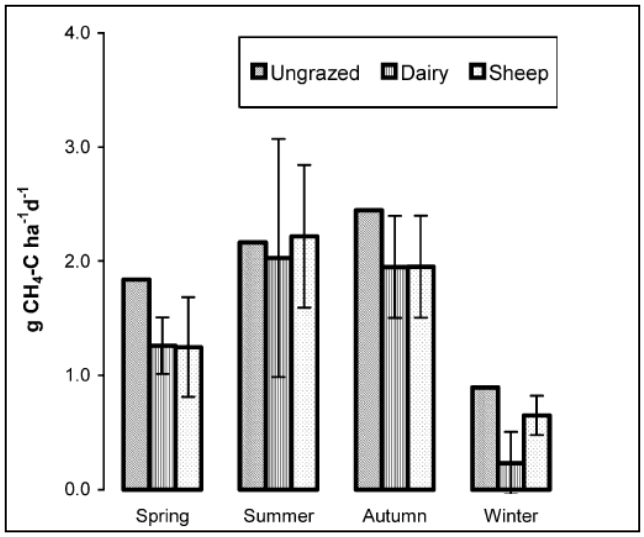

Figure 1.12. Seasonal pattern of soil methane sink in grazed (n=18) and ungrazed pasture (n=2) soils. Source: Saggar et al. (2008).

1.3.2.2.2. Soil N levels

Methane oxidation is lower in agricultural soils than in natural systems. Numerous studies have shown that the application of nitrogenous fertilizer to soils frequently inhibits methane oxidation (Bedard and Knowles, 1989; Bodelier and Laanbroek, 2004; Jollie and Lipscomb, 1991; King and Schnell, 1994). Two mechanisms to explain the inhibitory effect of nitrogen have been proposed: firstly, studies with pure cultures of methanotrophs show that ammonia is a competitive inhibitor of Methane MO. This enzyme oxidizes ammonia to hydroxylamine, which is then oxidized to NO_2^-, and ammonia will therefore reduce the amount of CH_4 consumed by methanotriphic bacteria in a concentration-dependent way. (Bedard and Knowles, 1989; Bodelier and Laanbroek, 2004). Secondly, it has been proposed that NO_2^- produced by methanotrophic nitrification is toxic to the organisms, possibly because it inhibits the last enzyme in the methane oxidation pathway, formate DH (Jollie and Lipscomb, 1991; King and Schnell, 1994). Inhibition of formate DH could starve Methane MO of the reductant required to metabolize methane. However, other studies have reported that N is essential. They support that NH_4^+ or NO_3^- relieves N limitation of cell growth and subsequently increases the activity of methanotrophic community in the long term. Also N addition interferes more directly with the synthesis of involved enzymes in the CH_4 oxidation pathway of nitrogen-starved cells and

increases the size and activity of the nitrifying population, which also oxidises methane (Bodelier and Laanbroek, 2004).

Published data from field experiments show a large variety of results. Mosier and Delgado (1997) observed different responses to fertilizer applications amongst types of soil with grassland: a small negative effect on CH_4 uptake in Vertisol, enhanced CH_4 uptake in the Ultisol and significantly decreased uptake in the Oxisol. Dobbie and Smith (1996), in Scotland, observed that the addition of N fertilizer to wheat soil did not have an immediate effect in the resulting oxidation rates (0.82 mg CH_4 m^{-2} d^{-1}) because the soil was already fertile but it affected the corresponding rates in a woodland soil (2.19-2.97 mg CH_4 m^{-2} d^{-1}). In a soil planted with maize, Collins et al. (2011), after excluding the time of slurry application, observed that the application of manure slurries lowered the seasonal uptake between 55-85% (12 and 37 g CH_4–C ha^{-1}) with respect to no fertilization. Jarecki et al. (2008) observed a decrease in CH_4 oxidation after swine manure slurry application to clay and sandy loam soils. They observed that the response of methane oxidation to N additions was moderated by soil type, with the effect being larger in the clay soil.

1.4. Carbon dioxide fluxes

The carbon cycle in an ecosystem initiates when plants fix CO_2 and water from the air to convert it into C organic compounds through photosynthesis with the help of photosynthetically active radiation. Environmental factors such as photosynthetically active radiation (PAR), soil moisture availability, air temperature, leaf area index and concentrations of CO_2 in the atmosphere influence the rate of photosynthesis (Gilmanov et al., 2003; Ruimy et al., 1995). Some of those organic compounds are used by plants to grow tissues (leaves, stems and roots) and other compounds are broken down to supply energy to plants, releasing CO_2 back to the atmosphere through respiration. Microorganisms decompose dead plant material (litter) to obtain energy for microbial growth and other activities and CO_2 is also released through microbial respiration. The live microbial biomass is mixed with organic residues of dead plants and dead microbes to form soil OM. This OM can be stored in the soil for hundred or thousand years before is broken down into CO_2 through respiration by microbes. Through the carbon cycle (Figure 1.13), CO_2 is produced by both plant respiration (R_p) and microbial respiration (R_m) that occurs during decomposition of litter and soil OM. Plant respiration is often called autotrophic respiration and can be separated into above-ground plant respiration (R_a) and below-ground plant respiration (R_b) (often equivalent to root respiration) and the rate of autotrophic respiration is associated with three major energy-requiring processes: growth, maintenance and

transport (Buchmann, 2000; Lambers et al., 1998). Microbial respiration (R_m) during the decomposition of litter and soil OM is called heterotrophic respiration. The heterotrophic respiration is controlled by soil temperature, soil moisture and substrate quality and reflects the microbial activity rate. The CO_2 rate measured at soil surface (R_s) is the sum of root respiration (R_b) and microbial respiration (R_m). Thus, the ecosystem respiration (R_e) can be estimated by sum R_a and R_s. Gross primary production (GPP) is the annual C assimilation by photosynthesis. Net ecosystem production (NEP) can be obtained by subtracting the gross primary production to ecosystem respiration, and also related to soil respiration by:

$$NEP = GPP-R_a-R_s \text{ (Eq. 1)}$$

Soil respiration is also related to net ecosystem respiration through net primary production (NPP), which is GPP minus autotrophic plant respiration, by the following equation:

$$NEP = NPP-R_m = NPP + R_b -R_s \text{ (Eq. 2)}$$

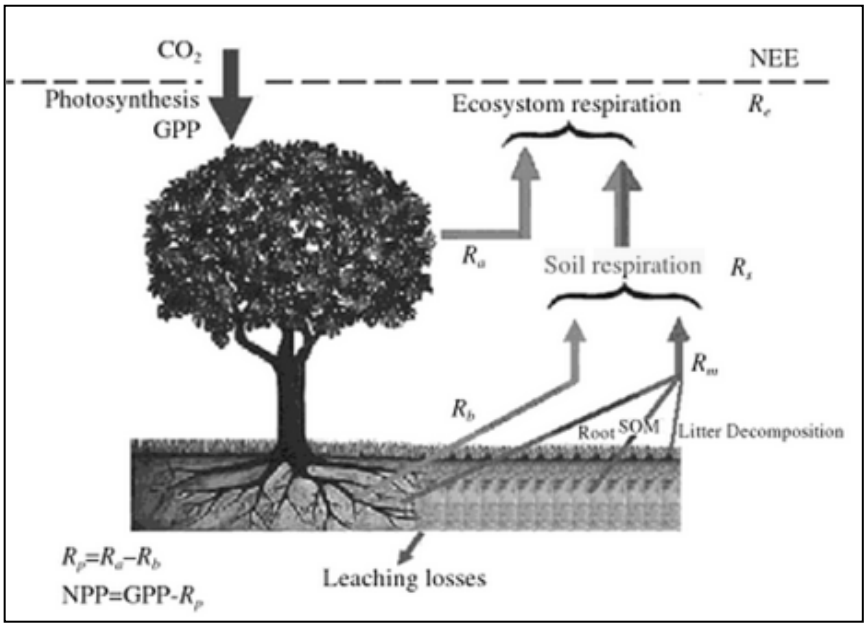

Figure 1.13. Schematic diagram of the ecosystem C process. Abbreviations are explained in the text. Source: Luo and Zhou (2006).

According to Houghton (2007), soil respiration in terrestrial ecosystems has been estimated to emit 58 Pg C yr^{-1} on a global scale but these emissions are balanced by plant photosynthesis which transfers approximately 59 Pg C yr^{-1}. Contrary, burning fossil fuels contributes to increased CO_2 emissions to the atmosphere (~6.3 Pg C yr^{-1}). Thus, soil respiration is one of the major flux pathways in the global

carbon cycle that could either mitigate atmospheric CO_2 emissions via increased C sequestration, or exacerbate global warming by accelerated release of CO_2 from soil.

Agricultural soils present an important reservoir of organic C. In agro-ecosystems, unlike forest ecosystems, the major C pool is located in the soil and not in the biomass. In soils the turnover is relatively slow, allowing the possibility of enhancement through management (Fischer et al., 1994). The amount of C stored in agricultural soils depends on climatic and site-specific conditions as well as on management decisions. Several studies have shown that it is not only theoretically possible, but practically feasible to regulate soil C stocks through improved management within upper and lower limits, which are determined by natural constraints (Ash et al., 1995; Batjes, 1999). Batjes et al.(1999) reviewed a series of land management options for enhanced soil C sequestration which included combinations of tillage methods and residue management (e.g. conservation tillage, cover crops, mulch farming), soil fertility and nutrient management (judicious use of fertilizers and organic amendments, improving NUE, nutrient cycling through cover crops and planted fallows, enhance biological N-fixation), water management (supplemental irrigation, surface and subsoil drainage, soil water management), erosion control (runoff management with vegetative barriers, soil surface amendment and mulch farming) and crop selection and rotation.

1.5. Methodology for gas measurement

Chamber is the most popular approach for measuring gas fluxes from the soil surface as they are easy to deploy and the technology is easy to adopt. Usually a sealable chamber or base is placed over the soil surface and closed for a period of time. Within that period of time gases build up and samples are collected to determine changes in their concentration over time. Chambers can cover an area up to 2 m^2 but the most common models have an area smaller than 0.5 m^2 so multiple chambers are required to account for the spatial variability of the gases emitted. Figure 1.14 shows some examples of equipment used to measure GHG emissions, which can be manually closed or opened or automated when they contain a pneumatic system. In the case of manual chambers, the number of readings that can be collected is limited but as they are cheap, more spatial locations can be sampled (Pattey et al., 2007). On the contrary, the use of automatic chambers allows many more readings over a longer period of study but because of their operating requirements and cost, this method is suitable for small areas. Chamber methodology guidelines and recommendations can be found in De Klein and Harvey (2012), Parkin and Venterea (2010) and Rochette (2011).

Figure 1.14. Automatic (round) and manual (square) chambers used in the experiments at North Wyke Research (left) and manual chambers at INGACAL-CIAM (right).

1.6. European dairy farming: the Galician case

The European dairy sector (EU-28) produces an estimated total of 151.1 million of tonnes of milk per year, 48% of which are produced in the countries as Ireland, UK, France, Belgium, Netherlands, Denmark, Spain and Portugal, all within areas located facing the Atlantic Ocean (Figure 1.15a) (FAOSTAT, 2013). This fact is reflected in the large percentage of utilised agricultural area (UAA) intended for feed production, which is in all cases larger than 70% (Figure 1.16). Within each of those countries, there is a region that leads the national production (Figure 15b). In Spain, the largest dairy producer region is Galicia, which contributes 39.2% to the national production (Figure 1.15b) and 1.7 % of the total milk produced in the EU-28 (FAOSTAT, 2013). Galicia is the Spanish region with the largest number of dairy cows (326,000 heads), 40.5% of the Spanish dairy census. The largest number of dairy cows and holdings intended to dairy production is mainly concentrated in the provinces of Lugo and La Coruña. According to Barbeyto Nistal and López Garrido (2013), the average Galician farm has 34.4 ha of UAA, 62.4 dairy cows, a stocking rate of 2.75 livestock units per hectare (LSU ha^{-1}) and annual production of 17,123 litres of milk per ha.

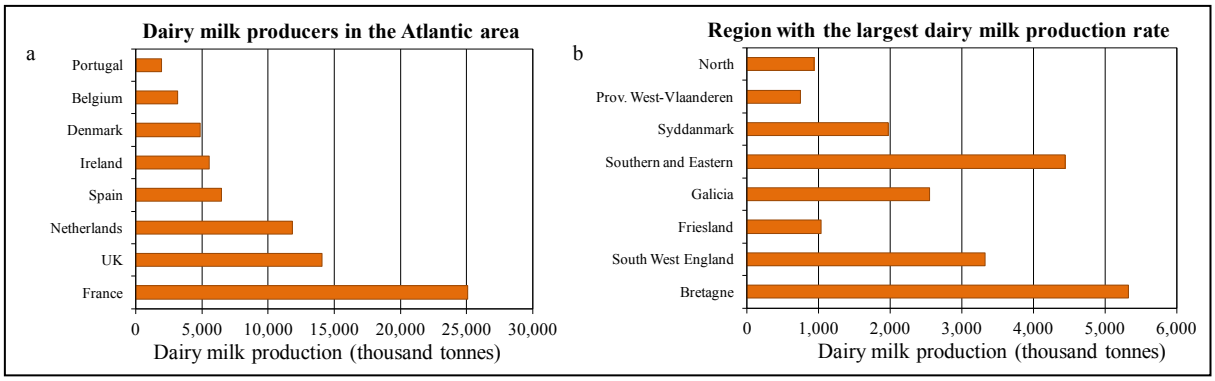

Figure 1.15. Dairy milk production in the Atlantic Area (a) and the largest producer regions at national scale (b) (EUROSTAT, 2013).

In terms of farming area, the land extent covered by permanent grasslands in Galicia is as large as in other important European milk producer regions, representing more than 50% of the UAA (Figure 1.16). The reason is attributed to its Atlantic climate (Fernández et al., 2011), characterized by high annual rainfall (>1000 mm) and cool temperatures. However, despite the large extension of grasslands in Galicia, fields are often very small and separated so grazing is difficult. This is in contrast with, for example, the full grazing and extensive system with limited supplemental feeding (up to 8 kg grass silage per cow and day when there is grass deficit in the paddocks) practice in Ireland. Also, with the English case where farms mainly practice restricted grazing (restricted number of cows graze during the whole day) (Reijs et al., 2013). To compensate for the absence of large land areas for grazing in Galicia, farmers feed livestock with concentrates (3.03 Tonnes per dairy cow and year, Barbeyto Nistal and López Garrido (2013)) and also forages (grass silage and maize). In Galicia, grasslands for grass silage represent 19.3% of the UAA (816,871 ha) much larger percentage than the percentage of 11.7% in the SW England (Figure 1.16). Also, forage maize is significant in Galicia, which occupies 8.4% of the UAA and 23% of the arable land (304,576 ha).

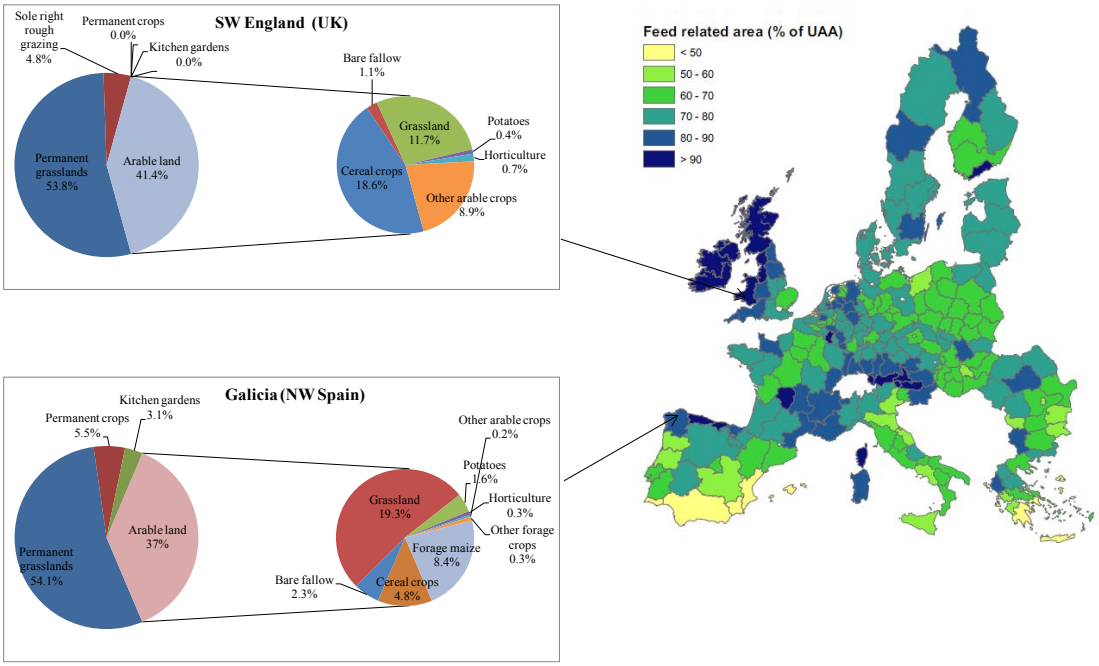

Figure 1.16. Land area utilized for animal feed production as percentage of total utilized agricultural area (UAA) within EU-27 and distribution of the utilised agricultural area (UAA) in Galicia and NW England. Sources: Lesschen et al.(2011a), ESYRCE (2013) and DEFRA (2009).

1.7. Legislation to reduce GHG emissions

On 11st December 1997, the United Nations Framework Convention on Climate Change, based on the reports produced by the IPCC about the impact of the anthropogenic activities on climate change, adopted an international agreement called The Kyoto Protocol which established legally binding limits for industrialized countries on emissions of CO_2 and other greenhouse gases derived from activities. The protocol, which entered into force on 16th February 2005, had concrete measurements, targets and timetables to achieve the reduction desired. The Kyoto Protocol has had two commitment periods: 2008-2012 and 2012-2020. To meet the targets established for 2020, the European Union follows the binding legislation included in the 2020 Climate and Energy Package, which aims to reduce GHG emissions by 20% relative to 1990, raise the percentage of energy consumption produced from renewable resources to 20% and improve energy efficiency by 20% in the EU. This legislation has complementary legislation, which will allow a reduction of 21% GHG emissions relative to the 2005 level for the more energy intensive and, under the EU Emission trading system (EU ETS), reductions of 10% less with respect to the same base year from the sectors not covered by the EU ETS, such as housing, agriculture, waste and transport (excluding aviation).

To ensure the compliance by Spain with its obligations with the Kyoto Protocol, the Spanish government activated two initiatives named "Spanish climate change and clean energies 2007-2012-2020" and "The National Plan for Climate Change Adaptation". The elaboration and development of both initiatives require the active participation of all Spanish Autonomous Communities, as well as the cooperation of the business sector and social organizations to achieve an efficient use of the energy. The collaboration of the Autonomous Communities is required as large part of the competences in the sectors or activities affected are transferred, enabling synergies between national and regional policies.

To help Spain to achieve the commitments agreed with the Kyoto protocol, the Autonomous Community of Galicia prepared its strategy against climate change. The first step was to elaborate an inventory of the GHG emissions to better understand the contribution of the main sectors in this region. This inventory was published in 2004 and contained the six main sectors contained in the Kyoto Protocol which were also broke down into more specific sub-sectors. The contribution of each sub-sector was estimated used the IPCC guidelines for greenhouse gas inventories.

The elaboration of the Galician inventory gave the first estimates of the environmental contribution of the agricultural sector in this region since 1990. According to the inventory, the estimation for 2012 reports a contribution of 11.1% (3,343.0 Gg eq CO_2) to the total GHG emissions in this region. The main causes are enteric fermentation, manure management and agricultural soils. In terms of type of gas, the main cause of the CH_4 emissions are enteric fermentation (61%) and manure management (39%). The largest sources of N_2O are agricultural soils (84%) and manure management (16%). Observing the estimations reported by the inventory, GHG emissions from agriculture clearly increased with respect to the base year (1990) (Figure 1.17). However, the contribution of the agriculture to the GHG is following a trend to decrease. The reason of this decline is largely due to the decrease in N_2O emissions from agricultural soils, which could be stimulated by the fluctuation in the price of mineral fertilizers and the economic crisis.

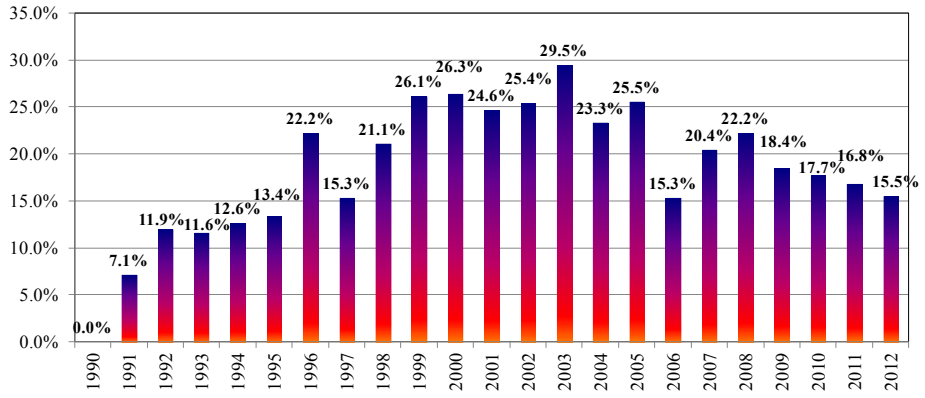

Figure 1.17. Variation of estimated GHG emissions from agriculture in Galicia respect to the base year (1990).

On 18th March 2005, the Government of Galicia (Xunta de Galicia) approved the Galician strategy against climate change, a document which proposes, measurements, actions and monitoring indicators that are intended to meet the effects of each sector on climate change, determine its consequences and identify solutions. The Galician strategy establishes specific course of actions for the agricultural sector as:

- Policies to promote compliance with the EU and national standards on sensitive areas, action plans, code of good agricultural practices, etc
- Conduct an inventory of sensitive areas and implement protection programs in these sensitive areas.
- Measures to support manure management and other livestock waste.
- Increase research on GHG emissions in the main agricultural activities.

1.8. Aim and objectives

Losses of N_2O, CH_4 and CO_2 derived from agricultural practices in dairy farms have been largely studied worldwide. In Europe, the environmental impact of dairy farming has been also largely documented and policies have been adopted to mitigate and reduce those losses, especially in those regions located in the Atlantic area where this sector has greater importance. In Spain, numerous studies about the impact of dairy farming in Northern areas have been developed and even from agricultural ecosystems influenced by Mediterranean climatic conditions. However, to date, the impact of dairy farming on losses of GHG in the NW Spain, where dairy farming is an important sector, is still unknown. It is important to address this knowledge gap because of the strong influence of soil and climate factors; factors that vary greatly from one region to another and have great influence on the magnitude and spatial and temporal variability of GHG fluxes.

The overall aim of this study was to evaluate the impact of the agricultural practices for dairy farming on losses of GHG from soils under the Atlantic climatic conditions in Galicia (NW Spain), in order to make recommendations for practices that reduce emissions and increase crop yields.

To address this main aim, a number of objectives were completed through five plot scale experimental studies. These were carried out between 2007-2011 at Mabegondo Agricultural Research Centre (CIAM-INGACAL) in Galicia (NW Spain) on grassland and forage maize soils. To complement these studies, an experiment was conducted in 2011 on a grassland site at Rothamsted Research, North Wyke, in Devon (SW England), in a contrasting soil type and management, under similar Atlantic climatic conditions as Galicia. The objectives were the following:

- To develop empirical models to explain inter-annual and seasonal variations from grazed and fertilized grasslands based on the measurement of the most influential parameters on N_2O emissions (Chapter 2).

- To quantify the effects of the application of mineral fertilizers and cattle slurry during spring, summer and autumn on GHGs from a grassland with grazing dairy cattle (Chapter 3).

- To investigate how typical management of slurry or mineral fertilizer affect GHG emissions in a grassland soil in the South West of England (Chapter 4).

- To understand the dynamics of the N_2O fluxes during the maize growing season under the Galician climatic conditions and determine the influence of the type of fertilizer (injected slurries and mineral fertilizers) typically applied by local Galician farmers on the resulting emissions (Chapter 5).

- To investigate the contribution of the different N fertilizers, commonly used in Galicia for forage maize cropping, on CH_4, CO_2 and N_2O fluxes in a short-term period after their application, quantify total N_2O fluxes derived from the whole maize grown season and provide yield scaled N_2O emission factors. (Chapter 6).

These experiments combined will also address three key hypotheses that are based on the following arguments:

I. <u>Inter-annual and seasonal variations in N_2O emissions.</u>

Losses of N_2O from soils are mainly caused by nitrification and denitrification processes, which are controlled by factors as soil moisture content, temperature, soil pH, organic carbon C and the availability of mineral N in the soil (Bouwman, 1996; Firestone and Davidson, 1989; Smith et al., 2003). Changes in these factors over time create considerable temporal variations in emissions of N_2O, making difficult to acquire representative estimates of annual N_2O emissions (Burchill et al., 2014). Large inter-annual N_2O variations from agricultural soils have been reported in other regions of the Atlantic area (Burchill et al., 2014; Cardenas et al., 2010; Dobbie et al., 1999; Flechard et al., 2007; Hyde et al., 2006; Rafique et al., 2011; Velthof et al., 1996). To investigate those variations, previous studies longer than 1 year were developed, and found that the cause of the large variation is generally attributed to climatic conditions in combination with N management (fertilization, grazing). As it was mentioned

above, there is a knowledge gap on how agricultural practices in this Spanish region affect losses of GHG from soils. Specifically, to date, there are not studies about inter-annual and seasonal variations of N_2O emissions from agricultural soils in this region. Based on the latter studies, it is expected that the combination of grazing and mineral fertilization can result in significant losses of N_2O from soils. In addition, grazing patterns, depending on the weather and soil conditions, can lead to large seasonal variability in N_2O emissions so it is necessary to increase the knowledge about the main factors that drive and affect N_2O emissions in each season to mitigate those losses.

We therefore hypothesise that combining grazing with fertilization under the climatic conditions of the NW Spain can lead in large inter-annual and inter-seasonal N_2O variations from soils, as in other regions of the Atlantic area.

II. Effect of fertilizer type on direct losses of N_2O and CO_2 from soils.

The use of organic fertilizers at farm scale can be a solution to reduce the cost of purchasing mineral fertilizers, minimizing the cost of milk production, and also an option to recycled animal wastes produced on farms. However, as discussed earlier, different types of fertilizer can have different effects on GHG emissions, so encourage the use of the fertilizer types that minimize emissions is a mitigation option. Slurries contain concentrations of NH_4^+-N and easily decomposable organic C that can, in concert, stimulate microbial activity and production of N_2O in soils (Rochette et al., 2000b; Velthof et al., 2003), which can be in some occasions larger than the resulting emissions after mineral fertilizer application. However, according to some studies N_2O emissions rates depend of the type of soil. In low C soils, N_2O emissions are related to easily decomposable organic C whereas in those with high C concentrations, N_2O rates are related to N availability (Chantigny et al., 2010; Pelster et al., 2012). **As Galician soils are generally rich in organic matter content, this thesis hypothesises that the addition of slurry-C to the soil would not significantly stimulate microbial activity and thus, would not increase CO_2 and N_2O production with respect to increases caused by mineral fertilizer applications as N_2O losses from soils rich in C are more dependent of the availability of N .**

III. The effect of fertiliser type and applications on CH_4 fluxes from soils.

Soil can be also a source or a sink of CH_4 and CH_4 fluxes from soils are the net result of methanogenesis, where CH_4 is produced in anaerobic environments with a source of C, and methanotrophy processes, where the CH_4 (produced in anaerobic parts of the soil and atmospheric CH_4) is consumed under aerobic conditions. Field experiments have previously

been carried out to test the effect of N fertilizer applications in soil CH_4 oxidation rates and diverse responses have been reported in the literature (Collins et al., 2011; Dobbie and Smith, 1996; Jarecki et al., 2008; Mosier and Delgado, 1997) from grasslands and arable soils. An interesting result was reported by Dobbie and Smith (1996), in Scotland, which reported no immediate effect in the observed oxidation rates to the addition to N fertilizer to wheat soil. They argued that it is the initial application of fertilizer to a soil that decreases CH_4 oxidation rates in that soil but there is not inhibitory effect from further N fertilizer applications **Based on this, we hypothesise that additions of N with organic and mineral fertilizers would not reduce soil CH_4 uptake capacity with respect to no fertilization in soils in Galicia that have received N fertilizer applications for many years.**

CHAPTER 2. INTER-ANNUAL AND INTER-SEASONAL VARIATIONS IN NITROUS OXIDE EMISSIONS FROM GRAZED AND FERTILIZED GRASSLANDS UNDER THE TEMPERATE HUMID CLIMATIC CONDITIONS IN NORTH-WEST SPAIN

Abstract

Data from a field study conducted in grasslands used for dairy production in NW Spain was used to develop five empirical models to predict temporal variations in the N_2O emitted. Nitrous oxide emissions from six grassland plots under different grazing and fertilization management and one with no nitrogen (N) inputs (control plot, CN) were frequently monitored between April 2007 and December 2009 using the closed chamber technique. The first model was developed to predict variations in the N_2O emitted at the annual scale ('inter-annual') and the other four at seasonal scale (winter: 'Jan-Mar', spring: Apr-Jun', summer: Jul-Sep' and autumn: 'Oct-Dec'). The five empirical models obtained were based on equations that better fit (Adj R^2) the following parameters (predictors): rainfall, mean air and soil temperatures, mean water-filled pore space (WFPS), soil ammonium (NH_4^+-N) and nitrate (NO_3^--N) contents, N fertilization rates and N excreted. For the 'inter-annual' model, data from January 2008 to December 2009 was considered. The mean annual N_2O-N emissions (kg ha^{-1}) were in the range 22.2-30.1 kg N ha^{-1} from managed grasslands and 12.5-13.6 kg N ha^{-1} from CN plot. The inter-annual model (2008-2009) showed that 24% of the variation in the N_2O emitted was caused by mean soil WFPS, rainfall, N fertilizer applied (N*fert*) and N excreted by livestock (N*excret*) (P<0.001). Other factors not included in the model and probably related to grazing (i.e. changes in soil bulk density, pH, available C contents) could have been responsible for the remaining variation. We did not obtain a significant model for the 'Jan-Mar' period, probably because of the lower frequency of the gas sampling during this season in 2008. However, significant models were obtained for the rest of the seasons. For 'Apr-Jun', 'Jul-Sep' and 'Oct-Dec' models, data from each period in 2007, 2008 and 2009 was used. Inter-seasonal variations in the cumulative N_2O emissions of 3.5, 2.7 and 2.0-fold were observed in 'Apr-Jun', 'Jul-Sep' and 'Oct-Dec', respectively. In 'Apr-Jun', 77% of the variation was explained by the combination of mean soil temperatures and N*fert* (P<0.001). In 'Jul-Sep', the combination of mean soil temperatures and mean soil NO_3^--N contents explained 72% of the variation (P<0.001). For 'Oct-Dec', 78.4% of the variation was attributed to differences in mean WFPS, rainfall, mean soil NH_4^+-N contents and N*excret* (P<0.001). We suggest that restricting the number of grazing hours per day and avoiding grazing when soil is very wet, especially during autumn, could help to reduce annual N_2O losses from dairy grasslands in the NW Spain. Future research is required to build models that are based on more detailed data related to grazing to allow for better prediction of inter-annual variation in N_2O.

2.1. Introduction

Nitrous oxide (N_2O) is an important greenhouse gas (GHG) which contributes to global warming and stratospheric ozone (O_3) destruction (Wuebbles, 2009). Nitrous oxide stays in the atmosphere for an average of 114 years and the potential for one molecule of N_2O to cause warming of the atmosphere is approximately 298 times greater than that of one molecule of CO_2 (IPCC, 2007). According to Smith et al. (2007), agriculture caused worldwide losses of 5.1-6.1 Gt CO_2 equivalents $year^{-1}$ in 2005 (10-12% of the total anthropogenic GHG emissions), 60% of which were caused by N_2O. Most of the N_2O is produced from agricultural soils and mainly due to two microbial processes: nitrification, which is the oxidation of ammonium (NH_4^+) to nitrite (NO_2^-) and nitrate (NO_3^-) under aerobic conditions and denitrification, which is the reduction of NO_3^- to nitrite (NO_2^-), nitric oxide (NO), N_2O and nitrogen gas (N_2) under anaerobic conditions. These two processes are responsible for approximately 56-70% of the global N_2O emissions (Braker and Conrad, 2011; Syakila and Kroeze, 2011). The timing and magnitude of N_2O emissions by these processes is mainly controlled by soil moisture content, temperature, soil pH, organic carbon C and the availability of mineral N in the soil (Bouwman, 1996; Firestone and Davidson, 1989; Smith et al., 2003). Changes in these factors over time create considerable temporal variation in emissions of N_2O on a seasonal and annual basis, making it difficult to acquire representative estimates of annual N_2O emissions (Burchill et al., 2014). Changes in these factors can be triggered by changes in rainfall patterns, temperatures, freezing and thawing, dry-wetting periods, management practices such as manure and fertilizer applications, grazing, incorporation of crop residues and tillage. Understanding these factors and how they influence N_2O emissions in a particular area is therefore a key step in developing mitigation options that reduce emissions.

In terms of grasslands, large inter-annual and seasonal changes have been reported from field studies in European temperate locations and predicted in empirical models (Burchill et al., 2014; Cardenas et al., 2010; Dobbie et al., 1999; Flechard et al., 2007; Hyde et al., 2006; Rafique et al., 2011; Velthof et al., 1996), but have yet to be investigated in Galicia, NW Spain, an important area of forage grassland. In this paper, a field study was conducted in Galicia, a region located at NW Spain where the dairy sector has large importance. This region produces 39.2% (2,55 millions of litres) of the annual volume of milk produced in Spain (EUROSTAT, 2013). Due to its climatic conditions (i.e., rainfall >1000 mm $year^{-1}$), grasslands represent 19% of Galician utilised agricultural area (810,871 ha) and 42 per cent of the Galician croppable land (374,916 ha) (ESYRCE, 2013). Nitrous oxide was monitored over 2 years and 9 months from N-fertilized and grazed grasslands. The data obtained was used to develop empirical models to explain inter-annual and seasonal variations from grazed and fertilized grasslands based on the most influential parameters for N_2O emissions.

2.2. Materials and methods

2.2.1. Location

The study was carried out at Mabegondo Agricultural Research Centre (CIAM-INGACAL), Abegondo, Galicia, Spain (43.2425°N, 8.2608°W). The climate is European Atlantic (Fernández et al., 2011) and Mediterranean humid according to agrologic classification provided by Papadakis (1966). The mean annual rainfall (1997-2006) is 1131 mm with autumn (October-December) being the wettest season (506 mm) and summer (July-September) being the driest (130 mm). The mean air temperature (1997-2006) is 13.3 ºC, with the lowest mean air temperatures of 9.4ºC occurring during winter (January-March) and the highest of 18.3 ºC occurring in summer. The soil is a free draining, acid brown earth with a silt-loam texture (27% sand, 56% silt and 17% clay, USDA classification system) and acid pH (5.5). At 0-30 cm depth, the soil was composed by 54.0 g kg^{-1} organic matter (OM), 2.5 g kg nitrogen (N), 18.2 g kg^{-1} phosphorus (P) and 195.9 g kg^{-1} potassium (K). Soil bulk density was 1.25 g cm^{-3} and pH of 5.6.

2.2.2. Experimental design

Six grassland plots (denoted as 1A, 1B, 2A, 2B, 3A and 3B, Figure 2.1), typical for dairy grazing in Galicia, were selected and monitored during 20th April 2007 to 14th December 2009 to identify temporal variability in N_2O fluxes. Plots were 0.5 ha and they were predominantly composed of perennial ryegrass (*Lolium perenne* L. cv. Brigantia) and less than 10% of white clover (*Trifolium repens* L.cv. Huia) which, after five years of grazing management, showed a low proportion of other species (less than 20%).

The grazing management used was the rotation system, allowing dairy cow livestock access to only one area of grassland at time and therefore providing resting periods for the rest of grassland. This system allows plant recovery and thus, maximizes production. To accomplish this system, before each grazing event, each grassland site was subdivided into 4 small areas and the livestock were moved from one small area to another.

In addition to the six grassland plots mentioned, an additional grassland plot was used as a control (CN), with similar size and composition, was also monitored during the same periods in 2007-2009. The CN grassland plot did not receive N inputs from fertilizers or grazing during the experiment and

also during the previous two years (2005-2006) to this experiment. However, CN plot received several grass cuts between 2007-2009: on 2nd March 2007, 14th May 2007, 28th June 2007, 10th August 2007, 26th September 2007, 26th December 2007, 10th March 2008, 14th May 2008, 1st July 2009, 23rd September 2009 and 24th November 2009.

Figure 2.1. Layout of grassland plots monitored between 2007-2009 at the experimental farm of CIAM-INGACAL.

2.2.3. Fertilization

Mineral N fertilizers were applied to each grassland plot (except in CN plot) by using a fertilizer drill, and the applied rate was according to individual requirements of each plot at the moment of fertilization. Several N applications occurred throughout the 2007-2009 period, registering annual rates of N in the ranges 130-169 kg N ha^{-1} in 2007, 77-115 kg N ha^{-1} in 2008 and 32-43 kg N ha^{-1} in 2009 (Table 2.1). Grasslands plots (including CN) also received annual quantities phosphorus (P) and potassium (K) to maintain the optimal soil fertility level (Table 2.1).

Table 2.1. Fertilizer applications dates (day/month) and rates (N:P:K, in kg ha^{-1}) and annual rates (N*fert*) applied to the grassland plots in 2007, 2008 and 2009.

		Grassland plot													
		CN		1A		1B		2A		2B		3A		3B	
Year	Application	Date	Rate	Date	Rate	Date	Rate	Date	Rate	Date	Rate	Date	Rate	Date	Rate
2007	1	21/03	0:102:102[5,6]	14/03	45:84:84[1]	14/03	45:84:84[1]	15/02	45:84:84[1]	15/02	45:84:84[1]	15/02	45:84:84[1]	15/02	45:84:84[1]
	2	24/05	0:88:88[5,6]	4/04	39:0:0[2]	4/04	39:0:0[2]	23/03	39:0:0[2]	23/03	39:0:0[2]	30/03	39:0:0[2]	30/03	39:0:0[2]
	3	2/10	0:61:61[5,6]	24/09	46:0:0[3]	24/09	46:0:0[3]	24/09	46:0:0[3]	24/09	46:0:0[3]	24/09	46:0:0[3]	24/09	46:0:0[3]
	4	-	-	-	-	-	-	-	-	-	-	5/10	39:0:0[2]	5/10	39:0:0[2]
Total	***N application***		**0**		**130**		**130**		**130**		**130**		**169**		**169**
	P application		**251**		**84**		**84**		**84**		**84**		**84**		**84**
	K application		**251**		**84**		**84**		**84**		**84**		**84**		**84**
2008	1	-	-	25/02	38:72:72[1]	25/02	38:72:72[1]	19/02	38:72:72[1]	19/02	43:86:130[4]	19/02	38:72:72[1]	19/02	38:72:72[1]
	2	-	-	12/05	39:0:0[2]	12/05	39:0:0[2]	23/04	39:0:0[2]	23/04	39:0:0[2]	23/04	39:0:0[2]	23/04	39:0:0[2]
	3	-	-	25/09	38:72:72[1]	25/09	38:72:72[1]	-	-	-	-	-	-	-	-
Total	***N application***		**0**		**115**		**115**		**77**		**82**		**77**		**77**
	P application		**0**		**144**		**144**		**72**		**86**		**72**		**72**
	K application		**0**		**144**		**144**		**72**		**130**		**72**		**72**
2009	1	29/05	0:101:101[5,6]	26/02	32:0:0[2]	26/02	32:0:0[2]	26/02	38:72:72[1]	26/02	43:86:130[4]	26/02	38:72:72[1]	26/02	38:72:72[1]
	2	28/09	0:66:66[5,6]	-	-	-	-	-	-	-	-	-	-	-	-
Total	***N application***		**0**		**32**		**32**		**38**		**43**		**38**		**38**
	P application		**167**		**0**		**0**		**72**		**86**		**72**		**72**
	K application		**167**		**0**		**0**		**72**		**130**		**72**		**72**

Mineral fertilizers applied: [1] 8:15:15 (N:P_2O_5:K_2O); [2] calcium ammonium nitrate 27% (CAN 27%); [3] granular urea; [4] 9:18:27 (N:P_2O_5:K_2O); [5] superphosphate 18% (18% P_2O_5); [6] potassium chloride (KCl)

2.2.4. Nitrogen excreted by livestock

Nitrogen excreted by dairy cow livestock in the grassland plots was estimated by using the indicator of the presence of the livestock grazing used by Bossuet et al. (2006). The calculation requires information about each single grazing event that happened in each grassland plot: livestock units (LSU), number of grazing days and number of hours per day grazing. Also, it was considered that, the daily N excretion was 0.2002 kg N cow^{-1} day^{-1}. This value, in agreement with the values reported in the literature for dairy cows (Aguerre et al., 2011; Burke et al., 2008; Spek et al., 2013) and a similar value was also obtained in a previous study carried out by Báez Bernal et al. (2008) at CIAM-INGACAL with dairy cows. In the later study, a mean annual N excretion per cow of 73 kg, was calculated from the balance between the N inputs during the livestock feeding (grass, silage and concentrates), N outputs (milk and meat) and also discounting the losses of N by ammonia (NH_3) volatilization.

Total N excretion (*Nexcret*) in each grassland plot during a single grazing event was estimated as follows (Eq.1):

$$N\textit{excret}\ (\text{kg N ha}^{-1}) = 0.2002 \times N \times LSU \times \text{number of grazing hours} \times 24\text{h}^{-1} \times A^{-1}$$

where *Nexcret* is the N excretion by livestock during a grazing event in kg N ha^{-1}, 0.2002 is the daily N excretion per cow (in kg N cow^{-1} day^{-1}) (Báez Bernal et al., 2008; Bossuet et al., 2006), N is the number of animals grazing, LSU is a constant that represents the type of livestock unit (dairy cattle: 1.00), number of grazing hours normalized to 24 hours and *A* is the area of the grassland plot (in ha).

2.2.5. Chamber design and operation

Nitrous oxide fluxes were monitored using the closed chamber technique (Louro et al., 2015). Chambers comprised cylinders of polyvinyl chloride (PVC) with a volume of 0.02 m^3 (height: 36 cm; diameter: 25 cm) and a PVC lid fitted to a septum rubber as a sampling port. To allow greater ground coverage of the spatial variability in N_2O fluxes resulting from the deposition of the urine and dung on the grassland plots studied, chambers were placed in different locations within each grassland plot (Hyde et al., 2006). The re-insertion into the soil (3 cm depth) was carried out after each sampling day. A total of four chambers per plot were used (i.e., 28 chambers per measurement day). On each sampling day, chambers were closed for 60 min (T_{60}). After 60 min, the chamber headspace was sampled through the rubber septum on the chamber lid. A sample of 12 ml from each chamber was

transferred to a 10 ml pre-evacuated glass vial using a syringe fitted with a hypodermic needle to over-pressurize the vials. Two ambient samples were also taken to provide N_2O background values and the mean value was used as the time zero sample (T_0).

2.2.6. Nitrous oxide flux measurements

Soil N_2O fluxes were measured 59 times between 20th April and 26th December 2007, 106 times between 8th January and 30th December 2008 and 97 times between 13th January and 14th December 2009. Except during the winter of 2008 and the autumn of 2009 where sampling frequency was lower, chambers were sampled up to three times a week, usually between 10:00 and 12:00 h. The N_2O concentration in each sample was analysed in the laboratory using a Thermo Finnigan Trace gas chromatograph (GC 2000) equipped with a HP-Plot Q column (30 mm × 0.3 mm) and an electron capture detector at 330ºC. Gas samples were injected into the GC in a split mode at a flow rate of 11ml min^{-1} and using helium (He) as a carrier gas at a flow rate of 1.5 ml min^{-1}. Calibration was performed using a 9.9 ppm standard of N_2O and the concentration of the gas in each sample was calculated by comparing the peak area of the samples and that obtained with the standard. On each sampling day, daily N_2O fluxes were calculated as follows (Eq.2):

$$F = \rho \times V/A \times (C_{60}-C_0)/t \times (273.15/T) \times 24 \times 10^4 \quad \text{(Eq.2)}$$

where F is the flux of N_2O (kg N_2O-N ha^{-1} day^{-1}), ρ the gas density (1.26 kg m^{-3} N_2O-N) under STP conditions (273 K and 101,325 Pa); V and A are the volume (0.02 m^3) and area (0.0491 m^2) of the chamber; C_{60}-C_0 (m^3 m^{-3}) are the N_2O concentration at T_{60} minus the concentration of the ambient sample (T_0); t is the time of chamber placement (60 min); and T is the air temperature (K) at the time of sampling. Cumulative N_2O emissions (expressed as kg N_2O-N ha^{-1}) were calculated using the trapezoidal method (Cardenas et al., 2010; Louro et al., 2013). Periods considered were selected based on the different seasons and they will be denoted as 'Jan-Mar', 'Apr-Jun', 'July-Sep' and 'Oct-Dec'.

2.2.7. Soil sampling and analyses

At each sampling day, four soil cores were taken to 10 cm depth within the area covered by each chamber, giving sixteen soil cores per grassland plot which were then bulked into one sample. After soil collection the chambers were randomly repositioned within the grassland plot. Soil samples were analyzed for ammonium (NH_4^+-N) and nitrate (NO_3^--N) contents and soil moisture to be transformed

to %WFPS. The methodology used for these analysis and the transformation of the gravimetric soil moisture into %WFPS is described in Louro et al. (2015).

2.2.8. Meteorological data

Total daily rainfall, daily mean soil and air temperatures were collected from 1st January 2007 to 14th December 2009 by the meteorological station located at CIAM. Average rainfall, air and soil temperature data for the previous 10 years (1997-2006) were used as a comparison for the weather conditions observed during the experiment.

2.2.9. Statistical prediction. Model development

2.2.9.1. Models and data

Measured and estimated data obtained during the experiment were used to develop five separate statistical models -that predicted N_2O emissions (Genstat version 15). The first model, denoted as 'inter-annual', was built to predict inter-annual N_2O emissions based on data from 2008-2009. Data from 2007 was not used for the inter-annual model as the period 'Jan-Mar 2007' was not measured. The other four models were built to predict inter-seasonal variations in the N_2O emissions. These empirical models were denoted as 'Jan-Mar', 'Apr-Jun', 'Jul-Sept' and 'Oct-Dec'. Except for the 'Jan-Mar' model where only data from 2008 and 2009 was used, the rest of empirical models contained data collected in 2007, 2008 and 2009. The data for each model were arranged in indexed format with independent variables total rainfall, total N*fert*, total N*excret*, and mean values for the soil mineral N (0-10 cm), soil temperature at 10 cm depth and air temperature. For the inter-annual empirical model, data from the seven individual plots within each separate year (2008, 2009) and period ('Jan-Mar', 'Apr-Jun', 'Jul-Sept' and 'Oct-Dec') were used, totalling 56 data points. For the four seasonal models, data from the seven individual plots across all three years were modelled separately for each period ('Jan-Mar', 'Apr-Jun', 'Jul-Sept' and 'Oct-Dec'). The data points for the each seasonal empirical models totalled 21, except for 'Jan-Mar' model which only had 14 points (absence of data for 2007). Prior to building the models, data was analysed for normality to assess if log-transformation was required. The result showed that most data fell within 95% limits of a normal distribution. Using Spearman rank correlation data was also plotted versus simple correlation and the test showed that all bivariate

relationships were linear and fell in 1:1 line. Based on this, data was not log-transformed prior to building the models.

2.2.9.2. Regression method and sensitivity analysis

A forward selection multiple regression procedure was used for the estimation of above prediction equations that maximised R^2 (Dhanoa et al., 1999). Further, the annual data was also used to investigate individual correlations(Pearson correlations) between cumulative N_2O emissions and the individual parameters described above.

A sensitivity analysis was performed with the parameters involved in the generation of the best-fitted inter-annual empirical model. To determine the sensitivity, the mean value of each parameter was calculated in order to obtain the mean N_2O emission. The mean of each parameter ±25% and ±50% was estimated and the resulting mean N_2O emission calculated while the remaining parameters were left unchanged. The percentage of change of each parameter was calculated by subtracting the mean N_2O emission to the resulting N_2O emission after increasing or decreasing 50% that parameter and dividing the resulting values by the mean N_2O emission.

2.3. Results

2.3.1. Weather conditions

Of the three years studied, 2007 was dry compared to 2008 and 2009. Total annual rainfall was 718, 1408, 1373 mm in 2007, 2008 and 2009, respectively. In 2007, the wettest period was 'Jan-Mar' (winter) and the driest 'Jul-Sep' (summer). The distribution of the rainfall during 2007 (expressed as % of annual rainfall) was 46% in 'Jan-Mar', 21% in 'Apr-Jun', 17% in 'Oct-Dec' and 16% in 'Jul-Sep' (Figure 2.2a). In both 2008 and 2009, the wettest period was 'Oct-Dec' (autumn) and the warmest 'Jul-Sep'. The rainfall distribution figures for each year were as follows: 34% in 'Oct-Dec', 27% in 'Apr-Jun', 25% in 'Jan-Mar' and 14% in 'Jul-Sep' in 2008 and 44% in 'Oct-Dec', 24% in 'Jan-Mar', 20% in 'Apr-Jun' and 13% in 'Jul-Sep' in 2009. In terms of air temperatures, 2007 was the coldest of the three years studied. Annual average temperatures were 12.7°C, 13.5 and 13.41°C in 2007, 2008 and 2009, respectively. The warmest period in all three years studied was 'Jul-Sep' with mean air temperatures of 16.9, 18.0 and 17.6 °C in 2007, 2008 and 2009, respectively (Figure 2.2a). In 2007 and 2008, 'Oct-

Dec' and 'Jan-Mar' were the coldest periods with mean temperatures of 9.8°C and 9.6°C in 2007 and 10.7°C and 10.1°C in 2008, respectively. In 2009, the differences between 'Jan-Mar' and 'Oct-Dec' were larger than in the previous years. The coldest period was 'Jan-Mar' (9.1°C), followed by 'Oct-Dec' (12.7°C). Mean soil temperatures in the periods studied were in the ranges of 8.4-18.7°C in 2007, 8.4-18.4°C in 2008 and 7.2-18.0°C in 2009, with 'Jan-Mar' and 'Jul-Sep' the coldest and the warmest periods, respectively (Figure 2.2a).

2.3.2. Soil WFPS and mineral N contents.

Mean soil WFPS (Figure 2.2b), NH_4^+-N (Figure 2.2c) and NO_3^--N (Figure 2.2d) contents from the seven grasslands monitored are presented in Figures 2.2b, c and d, respectively. In terms of WFPS, the mean values from grassland plots 2A and 2B (Figure 2.1) were in general larger than from the other five plots since the period Apr-Jun 08. We observed that in large number of sampling days these two plots had standing water at the soil surface (>100%WFPS) after rainfall episodes. Mean values obtained for the 'Apr-Jun' periods were 71% WFPS (range: 54-85% WFPS) in 2007, 90% WFPS (range: 74-115%WFPS) in 2008 and 82% WFPS (range: 69-104% WFPS) in 2009. In all years, the lowest soil WFPS contents were observed during 'Jul-Sep' as the mean values were 40% WFPS (range: 31-48% WFPS) in 2007, 55% (43-71% WFPS) in 2008 and 61% WFPS (range: 50-81%) in 2009. In 'Oct-Dec' periods, the mean soil WFPS levels were 56% WFPS (range: 50-64% WFPS), 77%WFPS (range: 69-90% WFPS) and 95% (range: 85-111%WFPS) in 2007, 2008 and 2009, respectively. In 'Jan-Mar', the corresponding means for 2008 and 2009 were 81%WFPS (range: 70-91%WFPS) and 90% (range: 75-103%), respectively.

Large and short -lived soil NH_4^+-N and NO_3^+-N peaks, compared to those from CN plots (no N inputs), were observed in this study when grazing and fertilization events were followed by soil sampling. Soil NH_4^+-N peaks were more frequent than NO_3^+-N peaks and also larger. In some occasions, soil NH_4^+-N peaks reached values of up to 200 kg N ha^{-1} while NO_3^+-N peaks were not larger than 100 kg N ha^{-1} (data not shown). Mean mineral N values used to input the models are shown in Figure 2.2c and 2.2d. In the six grazed grassland plots with N-inputs, mean soil NH_4^+-N contents were in the ranges 10-27 kg N ha^{-1} in 'Apr-Jun', 10-26 kg N ha^{-1} in 'Jul-Sep', 8-26 kg N ha^{-1} in 'Oct-Dec' and 3-18 kg N ha^{-1} in 'Jan-Mar' (Figure 2.2c). In the CN plot these ranges were 5-12, 9-11, 7-12 and 3-11 kg N ha^{-1} in 'Apr-Jun', 'Jul-Sep', 'Oct-Dec' and 'Jan-Mar', respectively (Figure 2.2c). Mean soil NO_3^+-N contents (Figure2.2d) were in the ranges 1-5 and 17-47 kg N ha^{-1} in 'Apr-Jun', 2-4 and 4-

18 kg N ha^{-1} in 'Jul-Sep', 3-4 and 4-21 kg N ha^{-1} in 'Oct-Dec', and 2-7 and 2-14 kg N ha^{-1} in 'Jan-Mar', for the CN plot and the six grassland plots under N-inputs, respectively.

2.3.3. Grazing data and N inputs

In 2007, the largest N-input source for the six grassland plots was through fertilization. Nitrogen excretion represented 43% (mean value) of the total N-inputs (Table 2.2). Although, in 2008, fertilization and grazing decreased by 31% and 23%, respectively, with respect to 2007, N*excret* still represented 48% (mean value) of the total N-inputs. In 2009, grassland plots received N mainly through livestock excretion (77% of the total N-inputs) and grazing was practiced throughout the year. The largest N*excret* in all years occurred during ’Apr-Jun'.

2.3.4. Nitrous oxide fluxes

Large and frequent N_2O peaks were observed immediately after grazing and fertilization events in all grasslands plots during the three years studied (Figure 2.3) and contrasted with those observed from the CN plot, with no grazing or fertilization events. In 2007, the daily mean N_2O fluxes from CN plot were 0.022, 0.010 and 0.016 kg N_2O-N ha^{-1} day^{-1} in 'Apr-Jun', 'Jul-Sep' and 'Oct-Dec', respectively. From grazed and fertilized grasslands, the corresponding fluxes (mean of the six grassland plots) for the same periods were 0.044, 0.035 and 0.034 kg N_2O-N ha^{-1} day^{-1}, respectively. In 2008, daily mean N_2O fluxes from the CN plot were 0.028, 0.035, 0.039 and 0.031 kg N_2O-N ha^{-1} day^{-1} in 'Jan-Mar', 'Apr-Jun', 'Jul-Sep' and 'Oct-Dec', respectively, lower than those of 0.075, 0.056, 0.048 and 0.053 N_2O-N ha^{-1} day^{-1} registered during the same periods from the remaining six grassland plots. In 2009, the lowest mean daily fluxes from CN plots during 'Jan-Mar', 'Apr-Jun', 'Jul-Sep' and 'Oct-Dec' were 0.020, 0.051, 0.044 and 0.037 kg N_2O-N ha^{-1} day^{-1}, respectively and 0.067, 0.123, 0.105, 0.066 kg N_2O-N ha^{-1} day^{-1} from the six grassland plots with N-inputs.

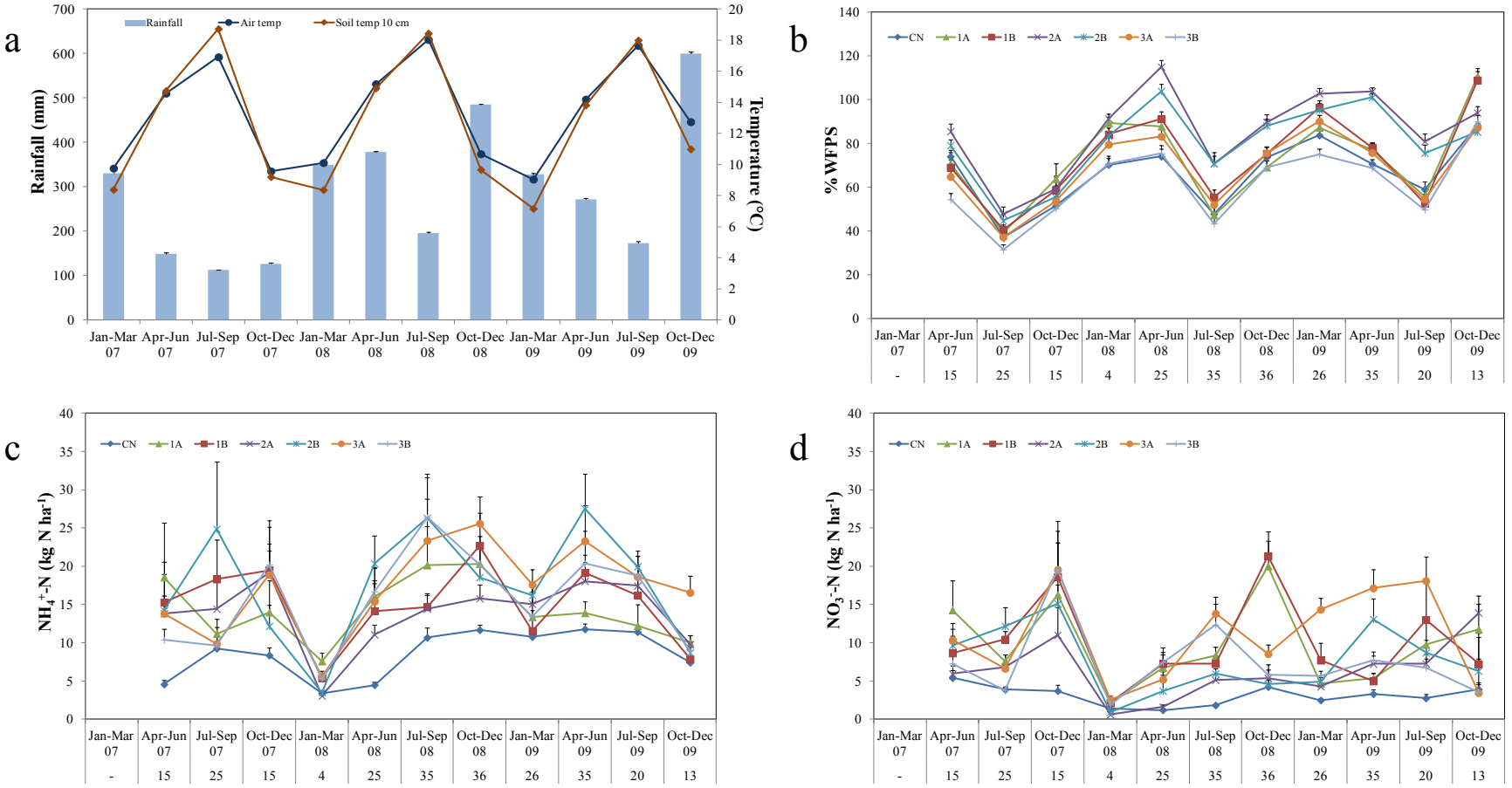

Figure 2.2. a) Total rainfall, and mean air and soil temperatures at 10 cm depth, b) mean soil WFPS, c) soil NH_4^+-N and d) soil NO_3^--N levels (0-10 cm) during the periods studied in 2007, 2008 and 2009. Data points represent the resulting mean values for each grassland after averaging all data values (number of sampling days indicated below periods) obtained in each period.

Table 2 2. Estimation of the N excretion (N*excret*, kg N ha^{-1}) during grazing and total N applied (N*fert*+ N*excret*, kg N ha^{-1}) at grassland plots monitored during 2007-2009.

		CN		1A		1B		2A		2B		3A		3B	
Year	Period	N*excret*	Total N	N*excret*	Total N	N*excret*	Total N	N*excret*	Total N	N*excret*	Total N	N*excret*	Total N	N*excret*	Total N
2007	Jan-Mar	0	0	28	112	28	112	14	98	24	108	36	120	24	108
	Apr-Jun	0	0	40	40	48	48	57	57	58	58	55	55	71	71
	Jul-Sep	0	0	31	77	44	90	6	52	38	84	14	60	34	80
	Oct-Dec	0	0	0	0	0	0	0	0	0	0	0	39	0	39
	Total	0	0	98	228	121	251	78	208	120	250	105	274	129	298
2008	Jan-Mar	0	0	0	38	0	38	0	38	0	43	0	38	0	38
	Apr-Jun	0	0	38	77	42	81	79	118	90	129	58	97	62	101
	Jul-Sep	0	0	17	55	10	48	0	0	10	10	30	30	7	7
	Oct-Dec	0	0	0	0	0	0	31	31	20	20	9	9	0	0
	Total	0	0	55	170	52	167	110	187	120	202	97	174	69	146
2009	Jan-Mar	0	0	25	57	21	53	12	50	27	70	21	59	34	72
	Apr-Jun	0	0	39	39	52	52	30	30	36	36	60	60	59	59
	Jul-Sep	0	0	19	19	28	28	34	34	18	18	31	31	23	23
	Oct-Dec	0	0	25	25	35	35	21	21	24	24	34	34	15	15
	Total	0	0	108	140	136	168	97	135	105	148	146	184	131	169

2.3.5. Cumulative N_2O emissions

During all of the periods studied between 2007-2009, the lowest cumulative N_2O emissions were observed from the CN plot (Table 2.3). In 2007, the largest losses were during 'Apr-Jun' (60% of the total N_2O losses). In 2008, during the periods 'Jan-Mar', 'Apr-Jun', 'Jul-Sep' plots emitted an average of 3.61 kg N_2O-N ha^{-1}. In 2009, a large proportion (39%) of the annual loss was also observed during 'Apr-Jun'. Grazed and fertilized grassland plots emitted an average of 8.78 kg N_2O-N ha^{-1} during the sampling period in 2007, 22.15 kg N_2O-N ha^{-1} in 2008 and 30.13 kg N_2O-N ha^{-1} in 2009 (Table 2.3). The largest cumulative N_2O emissions were in 'Apr-Jun' and 'Jul-Sep' during 2007 (average of 3.06 and 3.14 kg N_2O-N ha^{-1}, respectively), 'Jan-Mar' in 2008 (average of 6.90 kg N_2O-N ha^{-1}) and 'Apr-Jun' in 2009 (average of 10.64 kg N_2O-N ha^{-1}).

Table 2 3. Cumulative N_2O fluxes (kg N_2O-N ha^{-1}) during the periods studied in 2007, 2008 and 2009. Mean value of four chambers and standard error (between brackets).

	Plots						
Period	CN	1A	1B	2A	2B	3A	3B
Jan-Mar 07	-	-	-	-	-	-	-
Apr-Jun 07	2.57 (0.11)	3.36 (0.10)	3.22 (0.11)	2.70 (0.12)	2.57 (0.07)	3.05 (0.07)	3.46 (0.24)
Jul-Sep 07	0.80 (0.06)	3.38 (0.07)	3.70 (0.09)	2.76 (0.05)	2.89 (0.09)	2.78 (0.09	3.33 (0.19)
Oct-Dec 07	0.93 (0.01)	2.37 (0.10)	3.39 (0.05)	1.94 (0.05)	2.51 (0.40)	2.56 (0.11)	2.73 (0.08)
Total 2007	4.30 (0.06)	9.11 (0.26)	10.31 (0.18)	7.40 (0.14)	7.97 (0.50)	8.39 (0.12)	9.52 (0.41)
Jan-Mar 08	3.72 (0.04)	11.41 (0.79)	8.39 (0.16)	6.64 (0.10)	5.24 (0.17)	5.28 (0.21)	4.44 (0.14)
Apr-Jun 08	3.49 (0.01)	7.46 (0.12)	6.25 (0.15)	5.08 (0.07)	4.92 (0.04)	5.48 (0.15)	5.78 (0.06)
Jul-Sep 08	3.62 (0.01)	5.22 (0.05)	5.10 (0.08)	4.09 (0.06)	4.86 (0.06)	4.25 (0.07)	3.81 (0.06)
Oct-Dec 08	2.74 (0.02)	5.08 (0.04)	4.11 (0.04)	4.80 (0.05)	4.78 (0.10)	5.57 (0.18)	4.87 (0.02)
Total 2008	13.57 (0.06)	29.17 (0.88)	23.85 (0.40)	20.61 (0.17)	19.80 (0.17)	20.58 (0.21)	18.90 (0.20)
Jan-Mar 09	1.62 (0.00)	6.91 (0.03)	7.29 (0.14)	5.12 (0.05)	5.04 (0.03)	4.27 (0.06)	5.67 (0.16)
Apr-Jun 09	4.84 (0.00)	11.83 (0.07)	12.17 (0.04)	9.33 (0.04)	10.63 (0.13)	10.69 (0.12)	9.16 (0.01)
Jul-Sep 09	3.64 (0.01)	6.80 (0.06)	7.33 (0.15)	6.52 (0.15)	9.87 (0.05)	11.36 (0.02)	9.39 (0.07)
Oct-Dec 09	2.42 (0.07)	3.31 (0.19)	5.80 (0.17)	4.29 (0.24)	6.57 (0.07)	6.24 (0.11)	5.18 (0.21)
Total 2009	12.51 (0.07)	28.85 (0.30)	32.59 (0.12)	25.26 (0.32)	32.11 (0.15)	32.56 (0.05)	29.40 (0.39)

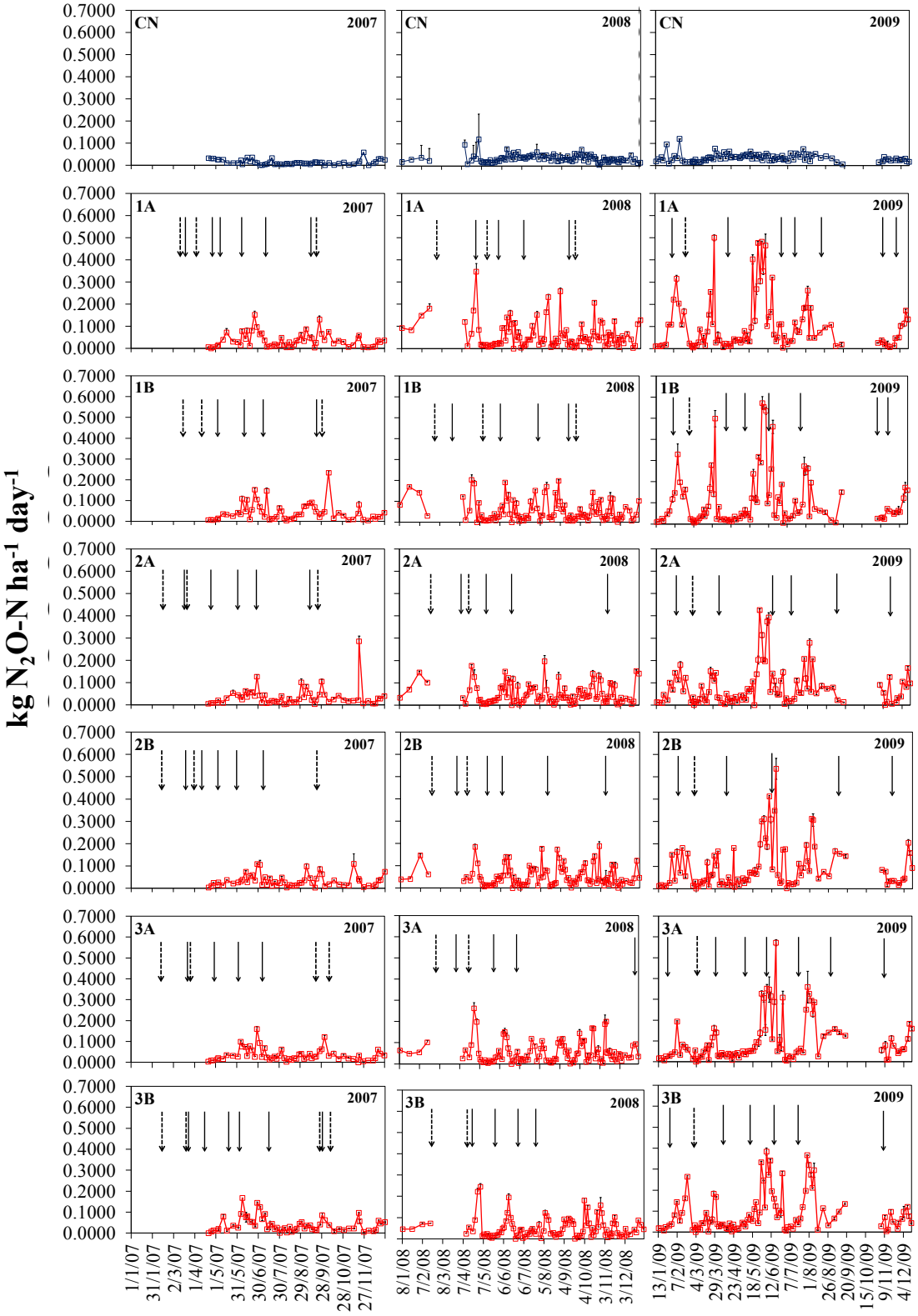

Figure 2.3. Daily N_2O fluxes observed from the grassland plots monitored in 2007, 2008 and 2009. Data points represent the mean value of four chambers ± standard error. Solid and dotted arrows denote grazing events and fertilization, respectively. Periods- 2007: (20th April-26th December); 2008: (8th January-30th December); 2009 (13th January-14th December).

Table 2.4. Pearson correlations between N_2O emissions and the variables for the inter-annual model

	N_2O	N*excret*	N fertilizer	Rainfall	Mean soil temp	Mean soil NO_3^--N	Mean soil NH_4^--N	Mean air temp	Mean WFPS
N_2O	-								
N*excret*	**0.3753** **	-							
Nfert	-0.0143	0.1742	-						
Rainfall	**-0.3241** *	-0.0186	-0.0606	-					
Mean soil temp	0.1642	0.2426	**-0.3044** *	**-0.596** ***	-				
Mean soil NO_3^--N	0.182	0.1366	**-0.3071** *	-0.0523	0.2046	-			
Mean soil NH_4^--N	0.244	**0.3459** **	**-0.277** *	-0.2929	**0.4029** **	**0.6047** ***	-		
Mean air temp	0.1514	0.2413	**-0.306** *	**-0.5611** ***	**0.9964** ***	0.1918	**0.3641** **	-	
Mean WFPS	0.0786	**0.387** **	**0.2685** *	**0.5734** ***	**-0.5446** ***	-0.0601	-0.2417	**0.5224** ***	-

* $P<0.05$; ** $P<0.01$; *** $P<0.001$

2.3.6. Empirical models to predict N_2O emissions

2.3.6.1. Correlations

Pearson correlations obtained with the data used to build the annual empirical model (Table 2.4) showed that cumulative N_2O emissions were positively correlated with N*excret*, and rainfall. The variable rainfall had also significant and negative correlations with air and soil temperatures and positive with mean soil WFPS. Significant and positive correlations were also observed between N*excret* with mean soil NH_4^+-N and mean soil WFPS. The rest of correlations between pairs are presented in Table 2.4.

2.3.6.2. Forward selection procedure for the inter-annual and seasonal models

Based on the forward selection procedure, the following 5-term model significantly predicts the resulting N_2O emissions for the inter-annual model:

N_2O emitted (in kg N_2O-N ha^{-1}) = 5.38 + 0.0318 × N*excret* (in kg N ha^{-1}) - 0.00944 × Rainfall (in mm) + 0.0456 × Mean WFPS (%) - 0.0239 × N*fert* (in kg N ha^{-1}) (Eq.3)

Using the same procedure, the best multiple regressions for the seasonal models were as follows:

Jan-Mar

N_2O emitted (in kg N_2O-N ha^{-1}) = -51.8 + 4.67 × Mean air temperature (in °C) + 0.1364 × Mean WFPS (in %) + 0.1212 × N*excret* (in kg N ha^{-1}) (Eq.4)

Apr-Jun

N_2O emitted = 107.2 - 7.046 × Mean soil temperature (in °C) + 0.096 × *Nfert* (in kg N ha^{-1}) (Eq. 5)

Jul-Sep

N_2O emitted = 148.3-7.90 × Mean soil temperature (in °C) + 0.1901× Mean soil NO_3^--N (in kg N ha^{-1}) (Eq. 6)

Oct-Dec

N_2O emitted = 2.99 + 0.00734 × Rainfall (in mm) + 0.1003 × Mean soil NH_4^+-N (in kg N ha^{-1}) + 0.0798 × N*excret* (in kg N ha^{-1}) - 0.0574 × Mean WFPS (in %) (Eq. 7)

The estimated coefficient values for the parameters included in the models and the corresponding standard errors and significance were summarized in Table 2.5. The variance explained by combining the parameters showed in each equation was 24.0% for the inter-annual model, 34.2% for the 'Jan-Mar ' model, 77.0% for the 'Apr-Jun' model, 72.0% for the 'Jul-Sep' model and 78.4% for the 'Oct-Dec' model. Except for the 'Jan-Mar' model, all models were significant at $P<0.001$.

2.3.7. Sensitivity analysis

Figure 2.4 shows the result of the sensitivity analysis for the inter-annual model. The inter-annual empirical model responded to changes in all the individual parameters included. However, cumulative N_2O emissions were more susceptible to environmental changes (specifically, rainfall and mean WFPS are both positively related, Table 2.4) than changes in N management (N*excret* and N*fert*).

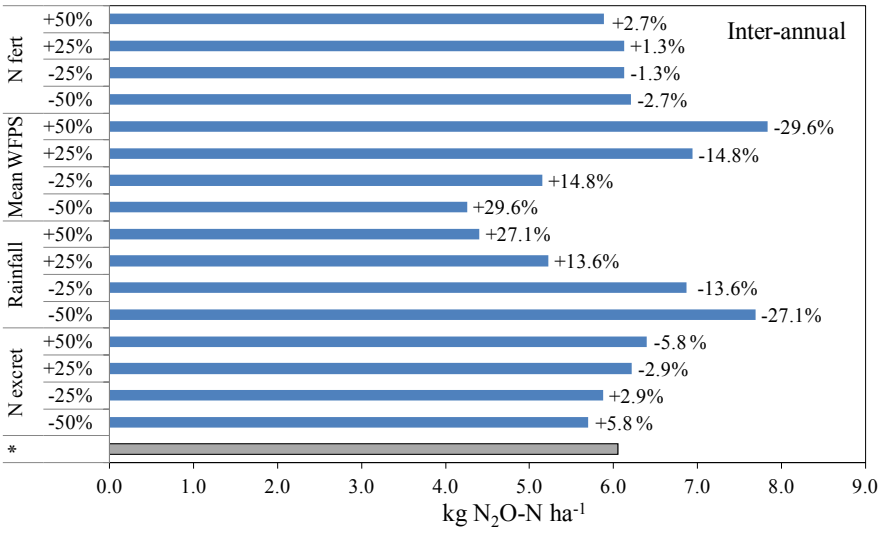

Figure 2.4. Effect of changing each parameter on sensitivity analysis for the inter-annual empirical model. Percentage values represent the percentage of variation of the N_2O emissions after changing each parameter respect to the baseline N_2O emissions (6.0 kg N ha^{-1}, denoted as *). Baseline values used for each parameter were: N*excret*: 22 kg N ha^{-1}; rainfall: 348 mm; mean WFPS: 78.5%; N*fert*: 14 kg N ha^{-1}.

Table 2.5. Estimated values for the final parameters that better predict cumulative N_2O emissions during the inter-annual model (2008-2009) and for the seasonal models ('Jan-Mar', 'Apr-Jun', 'Jul-Sep', 'Oct-Dec').

Inter-annual (2008,2009)	Parameter included in the model	Constant	N*excret*	Rainfall	Mean WFPS	N*fert*	*P* regression	Adj R^2
	Estimate value for parameters (se)	5.38 (1.44)	0.0318 (0.0154)	-0.00944 (0.00292)	0.0456 (0.0256)	-0.0239 (0.0172)	<0.001	0.240 (0.022)
	P	<0.001	0.045	0.002	0.041	0.170		
Jan-Mar (2008, 2009)	Parameter included in the model	Constant	Mean air temperature	Mean WFPS	N*excret*	-	0.077	0.325 (0.019)
	Estimate value for parameters (se)	-51.8 (19.4)	4.67 (1.73)	0.1364 (0.0629)	0.1212 (0.0707)	-		
	P	0.024	0.022	0.055	0.117	-		
Apr-Jun (2007, 2008, 2009)	Parameter included in the model	Constant	Mean soil temp	N*fert*	-	-	<0.001	0.770 (0.016)
	Estimate value for parameters	107.2 (12.2)	-7.046 (0.849)	0.096 (0.0232)	-	-		
	P	<0.001	<0.001	<0.001	-	-		
Jul-Sep (2007, 2008, 2009)	Parameter included in the model	Constant	Mean soil temp	Mean soil NO_3^--N	-	-	<0.001	0.720 (0.014)
	Estimate value for parameters	148.3 (26.9)	-7.90 (1.47)	0.1901 (0.0779)	-	-		
	P	<0.001	<0.001	0.026	-	-		
Oct-Dec (2007, 2008, 2009)	Parameter included in the model	Constant	Rainfall	Mean soil NH_4^--N	N*excret*	Mean WFPS	<0.001	0.784 (0.007)
	Estimate value for parameters	2.99 (1.38)	0.00734 (0.00164)	0.1003 (0.0320)	0.0798 (0.0188)	-0.0574 (0.0225)		
	P	0.045	<0.001	0.006	<0.001	0.021		

2.4. Discussion

2.4.1. Empirical models

We aimed to obtain inter-annual and seasonal empirical models for grassland soils in the NW Spain to predict the N_2O emissions based on the most influential variables in the emission of this gas. Six grazed and fertilizer grassland plots and one plot (designed as CN) with no N inputs were monitored between April 2007 and December 2009. Sampling frequency was high to provide more representative estimates of the losses of N_2O caused by the combination of grazing and fertilization under the climatic conditions in the NW Spain. As chambers were re-located after each sampling, the spatial variability of the N_2O emissions (caused by the heterogeneous distribution of the urine and dung patches) within each plot was also included in the measurements. We selected the equations based on the parameters that best predict N_2O (large Adj R^2). The parameters included in each equation were generally significant at $P<0.05$ and $P<0.001$. Only in a couple of cases these parameters were not significant ($P>0.05$) within a particular model, as it was observed during the inclusion of N*fert* in the inter-annual model or N*excret* in the 'Jan-Mar' empirical model (Table 2.5). However, they were included in the final models as their inclusion improved the significance of the rest of parameters and, in the case of the inter-annual model, improved the final Adj R^2 (Dhanoa et al., 1999).

2.4.2. Inter-annual variations in N_2O emissions.

Large inter-annual variations were observed in this study: there was up to a 1.4-fold difference in the cumulative N_2O emissions over two years (e.g. 2008 and 2009) from N managed grasslands. This difference would have been larger if data from the whole 2007 was used (excluded because there were no measurements in period 'Jan-Mar 2007'). Large inter-annual variations were also observed in other temperate European grasslands under grazing and fertilization, for example, 1.1-3.4- fold differences over two years in grasslands in England and Wales (Cardenas et al., 2010), a 20-fold difference over 3 years in Scotland (Dobbie et al., 1999), 1.1-3 fold differences over two years (Hyde et al., 2006; Rafique et al., 2011), up to a 7.8-fold over 4-years in Irish grasslands (Burchill et al., 2014) and up to a 5.8-fold over two years in Dutch grasslands (Velthof et al., 1996). Some of the factors that caused the inter-annual variation reported in the studies cited above were related to fertilizer N applications, grazing and weather conditions and their effect on soil conditions. In agreement with those studies, inter-annual N_2O emissions were driven by changes in the rainfall patterns, mean soil WFPS and N

management practices and explained 24% of the variation. In this sense, the inter-annual empirical model was obtained from wet years, although both had similar annual rainfall (differences of 2.6% in annual rainfall), there were differences in the seasonal distribution of the rainfall and thus, differences in the soil WFPS. When comparing all seasons among years we observed that the largest differences in cumulative N_2O emissions between these two years were during 'Apr-Jun' and 'Jul-Sep', with variations of 1.8 and 1.9-fold, respectively. In 2009, the year with the largest N_2O losses, the reduction in the annual N*fert* with respect to 2008 was supplied because of the increase in the grazing intensity and thus, N*excret*. That was reflected in the inter-annual model obtained by sign (positive/negative) of variables for those parameters (Table 2.5). In 2008, the bulk of grazing events were in 'Apr-Jun' and in 2009, although larger in 'Apr-Jun', grazing was practiced throughout all seasons in the managed grassland plots. The parameter N*excret* is related to the effect of the animals grazing on the soil as it was calculated based on number of animals, days, and hours per day grazing. In this sense, grazing occurred during large number of hours per day (18h), with no restrictions when the soil was very wet. Thus, the intense livestock treading when managed grassland plots were under those soil conditions could have caused more anaerobic microsites in the soil as consequence of soil compaction, which is in agreement with other studies (Drewry et al., 2008; Simek et al., 2006; Van Groenigen et al., 2005). The increasingly anaerobic conditions would have stimulated denitrification and thus, N_2O production. In addition, during grazing, large quantities of readily organic C contents were deposited on the soil from animal excreta, which would have also stimulated the denitrification process (Cameron et al., 1997; Chadwick, 1997; de Klein et al., 2001; Di and Cameron, 2003) and, therefore N_2O losses. The hydrolysis of urea in urine patches would have caused a decrease of soil pH, increasing the solubility of soil organic matter, availability of C and consumption by microorganisms which could also result in larger number of anaerobic microsites (Luo et al., 2013; Monaghan and Barraclough, 1993) available for N_2O production. The changes cited above caused by grazing and not included in the model (i.e. changes in soil bulk density, pH, available C contents) could explain inter-annual variation not explained for by rainfall, mean WFPS, N*fert* and N*excret*. Thus, we believe that restrictions in the number of grazing hours per day when soil is very wet (de Klein et al., 2006; Luo et al., 2008; Rafique et al., 2011) or even avoiding grazing (Van Groenigen et al., 2005) could minimize losses of N_2O.

2.4.3. Inter-seasonal variations on N_2O emissions

2.4.3.1. Winter periods ('Jan-Mar')

Some studies report large N_2O emissions during the winter. The reason of the losses observed during this season could be caused by periods of freezing and thawing because of the low temperatures. Burchill et al. (2014) observed in a white/clover grassland in Ireland that these episodes produced 39-fold more N_2O emissions in comparison with other winters where these cycles are missing. Syväsalo et al.(2004) in a two year study in boreal grassland also observed that these cycles contribute to increases of between 45-59% and 33-36% in N_2O emissions during winter periods, on a clay and a loamy sand soil, respectively. Lampe et al. (2006) and Kammann et al. (1998), both in Germany, reported that these episodes contributed 26% to the total cumulative 11-month emissions from a permanent grassland with a mixed managed system (cut and grazing) and 43-52% to the total annual N_2O emissions from grasslands with the highest amount of N fertilizer applied. These N_2O emissions are caused by the release of C and N from soil aggregates that rupture or microbial cells that lyse during the freezing periods and are then available for denitrifiers during thawing. It can also be because of the effect of the low temperatures on the N_2O/N_2 ratio due to the suppress of the N_2O reductase enzyme activity (Melin and Nômmik, 1983; Müller et al., 2002; van Bochove et al., 2000). In this study, we observed variations of 1.3-fold in the cumulative N_2O emissions during the winter but, contrary to the studies reported above, they were not related to those freezing and thawing cycles as daily temperatures did not reach temperatures below zero in any year monitored during winter. The model obtained for this season attributed changes in N_2O emissions to variations in soil WFPS, air temperature and Nexcret. During winters, optimal conditions for denitrification are caused by high rainfall and low evapotranspiration (Luo et al., 2000) and low growth state of the grassland (Saggar et al., 2004a). In this study, both winters had similar rainfall records (349 and 328 mm), which resulted in very wet soil moisture conditions. However, as air temperatures during winter 2008 were 0.6°C warmer than 2009, evapotranspiration was larger, resulting in less saturated soil conditions than in 2009. The inclusion of the Nexcret in the model would mean that if soil conditions were not that anaerobic during 2009, larger N_2O emissions would have been observed during 'Jan-Mar' 2009. In this sense, differences in evapotranspiration, which was not included in the model, would probably have played an important role during this period. However, the results of this model should be considered carefully as the resulting combination did not report significance, probably because of a possible overestimation in cumulative N_2O emissions caused by the lower sampling frequency during 'Jan-Mar' 2008.

2.4.3.2. Spring periods ('Apr-Jun')

Inter-seasonal differences in the cumulative N_2O emissions of up to 3.5-fold were observed during spring. from N managed grassland plots. During this season, air temperatures, and

consequently soil temperatures, rose up after the winter which, together with the frequent rain, early in the season, stimulates the growth of the pasture. This fact explains the required fertilizer applications and the large opportunity for grazing during this season. However, soil temperatures also stimulate and govern microbial processes in the soil and thus, N_2O production (Firestone and Davidson, 1989; Smith et al., 2003). This makes the spring one of the largest, if not the largest, contributing seasons to annual N_2O emissions. Soil temperatures were very similar in 2007 and 2008 but almost 1°C lower in 2009. However, the pattern of N_2O production was the opposite with greater emission in 2009. In 2007, soil WFPS conditions were optimal for both losses by nitrification and denitrification but the magnitude of the N_2O emissions would have been limited by the timing of fertilizer applications. The fact that most of the managed grasslands received N fertilizers late in the winter or early in spring (Table 2.1) before starting sampling measurements meant that N_2O losses related to fertilizer applications in spring 2007 could have been missed. In the springs of 2008 and 2009, denitrification would have been the main N_2O producing process and it would have been highly stimulated because of the increase of soil temperatures during the season. Dobbie and Smith (2001), for example, reported that at temperatures in the range of 12-18°C, denitrification showed Q_{10} values of 2.3 in a grassland soil. However, despite this stimulation, the process would have differed in the N_2O/N_2 rate of production amongst seasons. In 2008, although the soil conditions for losses of N_2O by denitrification were optimal, the larger N inputs from livestock and fertilizers than in 2009 would have decreased the N_2O/N_2 production ratio (de Klein and van Logtestijn, 1996), especially in those days where soil was saturated (in 58% of the days sampled WFPS>90%, data not shown) because of the high rainfall during the season in 2008 (39% larger than 2009). On the contrary, the rate of production would have increased in 2009 as soil WFPS presented levels between 60-80% in most of the days sampled (70% of the days, data not shown). The model revealed an effect of soil temperature and N fertilizer on N_2O emissions but not of soil WFPS, probably because of the decrease of N_2O emissions at low (as in 2007) and very high soil WFPS levels (as in 2008), which also explains the negative effect of the soil temperature in the model (largest N_2O emissions were in 2009 where soil temperature was almost 1°C lower compared to the other two years).

2.4.3.3. Summer periods ('Jul-Sep')

Large N_2O emissions are frequently observed during the summer months, in temperate (Hyde et al., 2006; Kim et al., 2010; Rafique et al., 2012; Rafique et al., 2011) and sub-tropical grasslands (Rowlings et al., 2015). Although this was the driest period of the year (113-196 mm of rainfall), large N_2O losses were observed when warm temperatures (18.0-18.7 °C) were

combined with wet conditions and available mineral N, causing inter-annual differences up to 2.7-fold in N_2O emissions. Hyde et al. (2006) associated large N_2O emissions during summer with fertilizer N applications that coincide with rainfall events. In this study, although no fertilization was made during this period in 2009, contrary to 2007 and 2008 (N applied late in the season), managed plots received N inputs through grazing. These grazing events coincided with times were rainfall was large and frequent, whereas in 2007 grazing occurred under drier conditions. The larger soil NO_3^--N contents was probably the cause, in addition to the wetter conditions, of the high magnitude of the resulting N_2O losses in this period in 2009. After grazing, peaks of NH_4^+-N were episodic and significant increases in soil NO_3^--N contents were not observed (data not shown), which probably denoted a rapid denitrification of the NO_3^--N formed. This was also supported by the levels of soil WFPS at the moment of the largest N_2O emissions, which were in the range 64-88%, optimal conditions for losses by denitrification. Some studies also attribute these losses to the combined effect of elevated soil temperatures, large N inputs and rewetting periods after a long period of soil dryness as in Kim et al.(2010). In this type of soil and region, dry-wetting periods have been observed by Louro et al. (2015) during the summer period in forage maize and elevated the resulting losses of N_2O from soil. In the latter study, the resulting fluxes were caused by an increase in a certain fraction of the soil OM (resulting from the death of the microbial population and from the disruption of soil aggregates) during the dry period which would be mineralized and N_2O emitted after soil rewetting. That could explain the losses of N_2O observed in all summers and also springs from the CN grassland plot (no N applications). This fact together with the more optimal conditions for N_2O production by denitrification during 2009 would have caused differences in the background values with respect to 2007 and 2008. However, the magnitude of the episodes of soil drying and rewetting were not as significant as those related to grazing.

2.4.4.4. Autumn periods ('Oct-Dec')

Under the contrasting weather conditions and the different N management, differences of up to 2.0-fold in cumulative N_2O emissions were observed during the autumn seasons. In 2007, only two grassland plots received N fertilizers in early autumn, in addition to those carried out late in summer. In this year, climatic conditions were drier than in the other two years and most of the rain occurred in this season from mid-November onwards (data not shown) and it was reflected in the low WFPS levels (<60%WFPS) early in the season. However, when soil WFPS conditions increased beyond this limit because of the frequency of the rain, only one significant peak of N_2O was observed from one of the N managed plots. This would have denoted larger plant N uptake in the managed plots early in the season, leaving less quantities of available

mineral N for possible losses by denitrification later on. In 2008 and 2009, N additions were through grazing. In 2008 only half of the N managed plots had livestock grazing while in 2009 all plots were grazed. Those events coincided with periods with frequent rainfall, which was more intense in 2009. This was also reflected in the soil. We observed that in both years WFPS levels were suitable for denitrification, however as Nexcret during 2009 was larger, losses of N_2O emission were 7.5% larger than 2008. This figure is considerably lower than the increase of 40-60% reported by de Klein et al.(2006) and Luo et al.(2008) when grazing with no restrictions is carried out under wet conditions in autumn. The reason of these differences would have been in the larger number of days with optimal conditions for complete denitrification during 2009 (in 2009, WFPS>80% in 77% of days versus 36% of days in 2008, data not shown), which together with the enhancement of anaerobic conditions caused by livestock grazing, resulted in the decrease in the N_2O/N_2 ratio of denitrification.

2.5. Conclusions

Understanding what factors and how those factors influence N_2O emissions in a particular area is a key step in developing mitigation options that reduce emissions. In Galicia (NW Spain), where dairy farming is an important sector, there were a knowledge gap about how N management practices under the influence of the Atlantic climatic conditions of this region affected N_2O emissions from grasslands. In this study, N_2O emissions were monitored over 2 years and 9 months from N-fertilized and grazed grasslands. The data obtained was used to develop five empirical models to explain inter-annual and seasonal variations from grazed and fertilized grasslands based on the most influential parameters for N_2O emissions. We observed large inter-annual variations in the N_2O emissions from grasslands used for dairy production, which were caused by variations in N management in combination with variations in weather conditions. Twenty-four percent of those inter-annual variations were explained by differences in rainfall, mean WFPS and N inputs (fertilization and excretion). As the largest annual N_2O emissions were observed when wet conditions were combined with unrestricted grazing daily grazing hours. we believe that other factors not included in the model and, probably, related to grazing (i.e. changes in soil bulk density, pH, available C contents) would have been responsible for the remaining variation. Variations in the N_2O emitted were observed in all seasons. High soil temperatures and N fertilizer application or available soil nitrate contents caused most of the variations observed in spring and summer. In autumn, the variations were caused by differences in rainfall records and the effect on soil WFPS as well as grazing and available ammonium contents in the soil. We suggest that restriction in the number of grazing

hours per day or even avoiding grazing when soil is very wet could reduce annual N_2O losses from dairy grasslands in the NW Spain. Future research is required to build models that are based on more detailed data related to grazing to allow for better prediction of inter-annual variation in N_2O.

CHAPTER 3. GREENHOUSE GAS (GHG) FLUXES FROM A GRAZED GRASSLAND AFTER CATTLE SLURRY INJECTION AND MINERAL FERTILIZER APPLICATIONS

Abstract

The dairy sector causes the largest annual greenhouse gas (GHG) emissions in Europe with the largest number of dairy farms located in the European Atlantic areas. The objective of this experiment was to quantify the effects of the application of mineral fertilizers and cattle slurry on GHGs from a grassland with grazing dairy cattle and identify the type of fertilizer associated with high grass production and low GHG fluxes. The experiment was located in Galicia, a region located in the Atlantic zone in North West Spain with humid climatic conditions (mean annual rainfall >1000 mm) and tempered temperatures (mean annual temperature of 13.2 ºC). Greenhouse gas fluxes were monitored during a seven-month experiment in 2011 after the spring, summer and autumn applications of mineral fertilizer (MN) and injected cattle slurry (CS) and they were compared with no fertilization (CN). In 2011, all seasons monitored suffered a reduction in the mean rainfall but it was especially important during spring and summer seasons where rainfall was very little. Dry soil conditions (<60%WFPS) were observed during the spring and summer, which was contrary to the higher soil WFPS (>60%WFPS) in autumn because of the more frequent rainfall. Total cumulative N_2O fluxes were similar in CS and MN (P>0.05). The largest cumulative N_2O fluxes were observed in the first 30 days after the spring application (as fertilization was followed by some rainfall) and autumn application. Short-lived CH_4 fluxes were observed immediately after each slurry injection and decreased in the following days to similar values as in CN and MN. Total cumulative CH_4 fluxes were significantly (P<0.05) larger from CS than MN and CN. The largest CH_4 fluxes from CS were observed after the application in autumn (P<0.05), probably because of the low dry matter content in the slurry in combination with wet soil conditions on the application day. Carbon dioxide fluxes were also short-lived after the three CS applications followed by a decrease to similar values as in CN and MN. However, these short-lived losses did not cause an overall difference in the total cumulative CO_2 fluxes respect to MN and CN (P>0.05). Overall, no significant differences between treatments were found when expressing total GHG (sum of N_2O, CH_4 and CO_2) losses in CO_2 equivalents (P>0.05), which were in the range 3.87-4.52 Mg equiv CO_2-C ha^{-1}. Yields scaled emission factors of 3.87 Mg equiv CO_2-C Mg DM^{-1} were obtained from CN, similar to 4.41 and 4.52 Mg equiv CO_2-C Mg DM^{-1} resulting from MN and CS treatments, respectively (P>0.05). Dry soil conditions during spring and summer would have stimulated organic matter mineralization, providing quantities of N that met crop demands. As both fertilizers caused similar total CO_2 equivalents to produce the same yields, both fertilizers could be used. However, if the costs of purchasing mineral fertilizers are considered, using injected slurries as fertilizer would be more beneficial for dairy farmers as animal wastes produced on farms would be recycled and milk production costs would be reduced.

3.1. Introduction

Grasslands are important in Europe as they cover approximately one third of the European agricultural area (Smit et al., 2008). They are used for animal feeding as they provide the nutrients required for domestic livestock to meet current demands of milk and meat quality. According to Smit et al. (2008), grasslands located in Atlantic areas are more productive than those in Mediterranean areas due to the frequent rainfall and cool temperatures. Thus, the largest number of dairy farms are located in these Atlantic Areas. According to Lesschen et al. (2011a), in comparison with other sectors (beef, pork, poultry and egg) the dairy sector causes the largest annual greenhouse gas (GHG) emissions (195 Tg CO_2 eq) in Europe. As the atmospheric concentration of these gases, (nitrous oxide (N_2O), methane (CH_4) and carbon dioxide (CO_2)), have increased between 1970-2010 (from 27 to 49 Gt CO_2 eq) and intensified in the decade 2000-2010 (2.2%) (Victor et al., 2014) policies should be adopted by institutions and countries to mitigate GHG emissions. Nitrous oxide is formed in soil by nitrification, where nitrifying microorganisms transform the ammonium (NH_4^+) contained in the soil in nitrate (NO_3^-) under aerobic conditions and by denitrification where NO_3^- is reduced to N_2O and, finally to N_2 gas under anaerobic conditions (Barton et al., 2008). Both processes are controlled by several factors as oxygen availability, soil water filled pore space (WFPS), mineral N content, temperature, available soil organic C and pH (Dobbie et al., 1999; Smith et al., 2003). Smith et al. (2003) reported that rainfall is the main controlling factor during the growing season in grasslands due to the influence of soil water status on N_2O production, especially if these events happen around the time of fertilization when large soil mineral N levels are available for losses of N_2O. In this sense, fertilization plans have to be adapted to crop demands as an excess of N in the soil can lead to large losses of N_2O. Grazing also results in large N_2O losses as the urine and dung deposited by grazing animals causes a high nutrient loading with respect to the small area intended for grazing and this high loading often exceeds plant N demand (Haynes and Williams, 1993; Zaman et al., 2013). In addition, the damage of the grass during intensive treading also causes the decrease of plant N uptake and therefore, accumulation in the soil of large amounts of N available for N_2O production. Livestock treading generates soil anaerobic environment by physical disturbance and compaction during grazing in combination with large inputs of N and C, which also stimulates denitrification (Luo et al., 1999; Menneer et al., 2005; Oenema et al., 1997; Saggar et al., 2004b; Saggar et al., 2011). When fertilizers and grazing are combined N_2O can reach annual losses up to 51.3 kg N ha^{-1} (Rees et al., 2013). Slurry application to grasslands is a frequent practice to increase grass yields in dairy systems as they are very valuable fertilizers and it is a solution to the large volume of animal waste generated in farms. Slurry application techniques such injection have been reported as an option to reduce losses of N by ammonia (NH_3) (Huijsmans et al., 2003; Misselbrook et al., 1996; Smith et al., 2000). However,

in some studies, the injection of the slurries seem to increased losses of N_2O respect other techniques (Ellis et al., 1998; Flessa and Beese, 2000; Wulf et al., 2002).

Grassland soils generally act as a sink of CH_4 via oxidation by methanotropic bacteria so short-lived CH_4 emissions are only observed immediately after manure applications to soil (Chadwick and Pain, 1997; Chadwick et al., 2000; Louro et al., 2013). Methane is the product of the organic matter decomposition carried out by methanogenic microorganisms under anaerobic conditions so once organic fertilizer is applied to the soil the diffusion of oxygen into the amendment on the soil surface inhibits CH_4 production (Chadwick et al., 2011).

Soils can also act as both source and sink of atmospheric CO_2. The CO_2 is fixed in plant biomass through photosynthesis and can be stored in the soil as organic C when plant residues are converted into soil organic matter. Respiration by plant roots and soil microflora and fauna also contribute a major portion of CO_2 emission from the soil (Curtin et al., 2000). Mineral and organic fertilizers can result in different responses in soil CO_2 fluxes as Rochette and Gregorich (1998) reported after mineral or slurry applications. They observed that the application of slurries can increase 2.2-2.6-fold CO_2 fluxes with respect to no fertilizer due to the increased in soil respiration and levels of soluble organic C and microbial biomass C, whereas mineral fertilizers had little effect on these parameters. It is therefore important to identify the type of fertilizers that minimize GHG emissions in the specific soils and climates of Europe.

In Spain, agriculture causes 84.1% and 53.9% of the anthropogenic N_2O and CH_4 (MAGRAMA, 2015). The dairy sector is concentrated in the northern areas where the largest extension of grasslands are located. In Galicia, a region located in the Atlantic zone, grasslands represent 19% of the utilised agricultural area (816, 871 ha) and 42% of the croppable land (374,916 ha) (ESYRCE, 2013), which means that this region is one of the main dairy milk producers in Spain. Despite this, there is currently little research into the natural or human factors that influence GHG emissions in Galician grasslands. Based on this, the objective of this study was to quantify the effects of the application of mineral fertilizers and cattle slurry during spring, summer and autumn on GHG fluxes from a grassland with grazing dairy cattle. Based on the yield-scaled emission factors (expressed as total CO_2 equivalents per unit of dry matter yield), we aim to find the type of fertilizer (mineral fertilizer/ injected cattle slurry) that increases grass production without increasing GHG fluxes.

3.2. Material and methods

3.2.1. Experimental site and soil characteristics

A field experiment was carried out between 13th April and 8th December 2011 in a grassland located at Mabegondo Agricultural Research Centre (CIAM-INGACAL), in Abegondo, Galicia, Spain (43.2425°N, 8.2608°W). The pasture consisted of a mixture of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.). The soil is a Humic Cambisol with a silt loam texture (USDA classification). The bulk density (BD) at 0-10 cm soil depth was 1.07 g cm^{-3} and the pH (H_2O) value was 5.7. Total C, total N and organic matter were 4.8%, 0.42% and 10.4%, respectively. The climate is European Atlantic (Fernández et al., 2011) with annual mean rainfall and air temperatures (2001-2010) of 1173 mm $year^{-1}$ and 13.2ºC, respectively. The agroclimatic classification of the area where the experiment was located is Mediterranean Maritime according to Papadakis (1966).

3.2.2. Experimental design

A randomized block design was set up with three replicate plots of the following treatments: control with no N fertilizer (CN), mineral N fertilizer as CAN 27% (MN) and cattle slurry injected into the soil at 5 cm depth (CS). Each replicate plot was 0.15 ha in area (42.5 m x 35.0 m).

3.2.3. Fertilization events and characteristics of mineral fertilizer and slurries applied

Treatments were applied three times during the study: 13th April (spring application), 14th June (summer application) and 8th November (autumn application). Mineral fertilizer was applied as calcium ammonium nitrate 27% (CAN 27%) at rates of 36, 45 and 40 kg N ha^{-1} in the spring, summer and autumn applications, respectively using a fertilizer spreader. Phosphorus (P) and potassium (K) fertilizers were applied in CN and MN treatments to ensure that these nutrients would not limit herbage production and also to equal the amounts of P and K applied with the slurry. Phosphorus and potassium fertilizers were applied on the 13th April and 8th November using granular simple super phosphate 18% and potassium chloride 60%. Rates of 28 and 30 kg ha^{-1} of P (as P_2O_5) and 121 and 67 kg ha^{-1} of K (as K_2O) were applied in the spring and autumn applications, respectively.

Cattle slurry was collected from the pit located at CIAM-INGACAL. The pit was sampled in the previous months to this study for the analysis of N contents in the slurry and calculation of N to be applied in the experiment. The slurry samples taken showed that 56% of the total N was as mineral N and entirely as ammonium (NH_4^+-N) (no nitrate, NO_3^--N, was detected). Based on this and with the aim to equal N rates in the MN treatment, slurries were injected at 5cm depth with target rates of 26, 35 and 28 m^3 ha^{-1} in the spring, summer and autumn applications, respectively. On each application day, slurry samples were collected from the slurry injector tank and analysed to determine the chemical composition of the slurries applied (see section 2.8). These analyses showed that the NH_4^+-N contained in the slurries used in spring and summer slightly varied respect to previous analysis. The final N rates applied were those reported in Table 3.1.

Table 3.1. Amounts of the different N compounds incorporated to the soil (in kg N ha^{-1})as mineral and cattle slurry. Treatments- CS: cattle slurry; MN: mineral fertilizer; CN: control.

Fertilization	Date	Treatment	Total N	NH_4^+-N	NO_3^--N	N org
Spring	13th April	CS	83	46	0	37
		MN	36	18	18	-
		CN	-	-	-	-
Summer	14th June	CS	108	61	0	47
		MN	45	22.5	22.5	
		CN	-	-	-	-
Autumn	8th November	CS	67	38	0	29
		MN	40	20	20	-
		CN	-	-	-	-

3.2.4. Grazing events and N excreted by livestock

A rotational grazing system was used during the experiment. This practice can control the paddocks where forage growth is at peak production (high forage quality and easy digestion). Also, it allows resting periods between grazing events which help to maintain healthy forages since rotational grazing controls the production of weeds and undesirable plants which often invade overgrazing areas. Blocks were rotationally grazed one by one and livestock was not allowed to access to the next block until the previous block was completed grazed. Within each

block, treatment plots were simultaneously grazed. The herd size was divided into three groups of a mean of 11 animals per group. Each treatment plot was divided into three small areas to allow the rotational grazing. Different groups of animals (dairy cattle, pregnant cows and heifers) were used to graze the experiment a total of five times during the experiment, which are shown in Table 3.2.

Total nitrogen excreted by livestock (Nexcret) in each grazing event was estimated as follows ((Eq.1)):

$$N\textit{excret}\ (\text{kg N ha}^{-1}) = 0.2002 \times N \times LSU \times \text{number of grazing hours} \times 24\text{h}^{-1} \times A^{-1}$$

where *Nexcret* is the N excretion by livestock during a grazing event in kg N ha^{-1}, 0.2002 is the daily N excretion per cow (in kg N cow^{-1} day^{-1}) (Báez Bernal et al., 2008; Bossuet et al., 2006), N is the number of animals grazing, LSU is a constant that represents the type of livestock unit (dairy cattle: 1.00; pregnant cows and heifers: 0.85), number of grazing hours normalized to 24 hours and *A* is the area of the grassland plot (in ha).

Table 3.2. Grazing events during the experiment and N excreted (N*excret*, in kg N ha^{-1}) in each grazing event and treatment. Values represent the mean value of three blocks with the standard error (in brackets).

Grazing event	Period grazing	N	Number of grazing hours	LSU[a]	N*excret*
1	2nd to 10th May	13	47 (6)	1.00	34 (4)
2	7th to 11th June	13	38 (7)	0.85	24 (5)
3	27th July to 5th August	13	48 (0)	1.00	35 (0)
4	26th September to 3rd October	10	55 (7)	0.85	26 (3)
5	12th to18th December	7	48 (0)	0.85	16 (0)

[a] *LSU*: livestock unit

3.2.5. Chamber design and operation

Greenhouse gas (N_2O, CH_4 and CO_2) fluxes were measured using a closed chamber technique (Louro et al., 2015). Chambers were comprised by a cylinder of polyvinyl chloride (PVC) with 24.02 l volume (diameter 25 cm, height 36 cm) and a PVC lid fitted with a sampling port with a three-way valve. Chambers were inserted in the soil 24 h before starting gas measurements . Six chambers per replicate plot were used (i.e., 18 chambers per treatment). Chambers were returned to the same position after each grazing event and fertilizer application. The effective height of each chamber above the ground (H) was measured internally in the centre of the

chamber. The resulting effective chamber height ranged between 23-27.5 cm. Values of chamber height recorded were used to calculate GHG fluxes from each chamber after insertion in the soil. Gas measurements were usually carried out between 10:00 and 13:00 h. On each sampling day, chambers were closed for 50 min. After 50 min (T50), the chamber headspace was sampled via the three-way valve on the chamber lid. A sample of 60 ml of the chamber headspace was taken and transferred to a 20 ml pre-evacuated glass vial using a syringe and two hypodermic needles: one to inject the sample into the vial and the second one to release the sample to ambient pressure. Two ambient air samples were taken and the average was used to provide a background value (T0). Two digital thermometers were used to record soil and air temperatures in each chamber.

3.2.6. Greenhouse gas flux measurements and laboratory analysis

Fluxes of N_2O, CH_4 and CO_2 were measured immediately after first fertilization carried out in spring (from 13th April to 6th June), immediately after the second fertilization in summer (14th June to 20th July 2011), and before and after the third fertilization in autumn (from 6th October to 21st December 2011). Gas samples were analysed with an Agilent Technologies gas chromatograph (Agilent 7890A) equipped with a capilar column (IA KRCIAES 6017: 240 ºC, 30 m x 0.32 mm) and two detectors: ^{63}Ni electron capture detector (ECD) at 300ºC for measuring N_2O and a flame ionization detector (FID) at 300ºC for CH_4. For CO_2 determination, the GC was equipped with a methanizer to reduce CO_2 to CH_4. The injection was carried out with a six pot valve. Gas standards (0.22, 0.33, 0.88, 1.10, 8.9 ppm for N_2O; 1.67, 8.4, 16.7 ppm for CH_4; 399, 1995, 3990 ppm for CO_2) were used for calibration and to calculate the concentrations of gases in the samples by comparing peak areas integrated with those obtained with the standards of each gas.

Daily greenhouse gas fluxes were calculated from the accumulation of each gas in the chamber as follows ((Eq. 2)):

$$F = \rho \times H \times (C_{50}-C_0)/ t \times 273.15/ T \times 10^4 \times 24 \quad \text{(Eq.2)}$$

where F is the gas flux (kg N_2O-N ha^{-1} day^{-1}, g CH_4 ha^{-1} day^{-1}, kg CO_2-C ha^{-1} day^{-1}), ρ is the gas density (N_2O-N: 1.26 kg m^{-3}; CH_4: 717 g m^{-3}; CO_2-C: 0.536 kg m^{-3}) under STP conditions (273.15 K, 101,325 Pa), H is the effective height of the chamber (m), C_{50}-C_0 is the gas concentration at 50 minutes (T50) after chamber closure minus gas concentration of ambient sample (T0) (μm^3 m^{-3}), t is the time of chamber closure (50 min) and T the temperature (K) inside the chamber. Total cumulative fluxes of N_2O, CH_4 and CO_2 fluxes (expressed as kg N_2O-N ha^{-1}, kg CH_4 ha^{-1} and Mg CO_2-C ha^{-1}) were calculated for each period considered and during

the whole experiment by using the trapezoidal method (Cardenas et al., 2010; Louro et al., 2013). In the period between 21th July and 5th October no measurements were taken. Mean cumulative GHG fluxes were also calculated in different periods after fertilizations. Factors of 298, 25 and 1 were used to convert total N_2O, CH_4 and CO_2 fluxes in total CO_2 equivalents (IPCC, 2007). The value obtained was used for the calculation of total CO_2 equivalents emitted per unit of DM yield produced in each treatment. For N_2O, emission factors (expressed as %) based on the loss per kg of N applied with fertilizers ($EF_{Napplied}$) and per kg of N uptake by plant in each treatment ($EF_{Nuptake}$, Schils et al. (2008)) were calculated.

3.2.7. Soil sampling and analysis

Once a week, six soil cores from the 0-10 cm soil profile were collected from each single plot for analysis of gravimetric soil moisture and mineral N (ammonium, NH_4^+-N and nitrate, NO_3^--N) as it was described in Louro et al. (2015). Soil bulk density (BD) was determined at the start of the experiment. Three metal rings (3.8 cm diameter, 8cm height) were used for the sampling. The soil sample contained in the ring was dried at 105ºC to constant weight. Bulk density was finally calculated from the dry soil weight and the volume it occupied and expressed as Mg m^{-3}. Total soil porosity was calculated by the following equation (3):

$$Porosity = 1 - (BD/2.65) \quad (Eq.3)$$

where BD is the soil bulk density (Mg m^{-3}) and 2.65 is the assumed particle density value (Mg m^{-3}). Water filled pore space (WFPS), expressed as percentage, was calculated by multiplying the gravimetric soil moisture by BD and dividing by total soil porosity (Louro et al., 2015).

3.2.8. Slurry analysis

Density, pH, dry matter (DM), total N, total P, total K and total C were determined by using the methodology described in Louro et al. (2015). Organic matter (OM) was analysed in dry and ground (<0.01mm) samples using a thermogravimetric analyzer TGA-601 (LECO Co., Michigan, USA). Chemical and physical properties of the cattle slurry applied to the soil are summarized in Table 3.3.

Table 3.3. Characteristics of the dairy cattle slurries used in this experiment.

		Dairy cattle slurry applications		
		Spring application	Summer application	Autumn application
Properties	Units	13th April	14th June	8th November
Density	g cm^{-3}	1.09	1.07	1.05
pH	-	8.45	8.45	8.39
DM	%	7.5	6.9	6.9
OM	% DM	80.7	79.1	76.2
Total P	% DM	0.56	0.61	0.67
Total K	% DM	5.44	5.48	6.79
Total N	% DM	3.91	4.20	3.31
Total C	% DM	39.93	39.21	38.48

3.2.9. Grass sampling and analysis

Grass samples were taken prior to grazing events on 2nd May, 9th June, 26th July, 21st September and 12th December 2011. For every single grass sampling, three samples per plot were taken using a square of 0.25 m^2 (0.5m x 0.5 m) and a knife. Grass samples were analysed for DM and total N. Dry matter was determined by oven-drying the samples for 24-36 h at a temperature of 70ºC. Total N was determined in dry and ground samples by Kjeldhal (AOAC, 2000). Both parameters were used to calculate DM yields and N plant uptake. With the total N harvested during the experiment from each N treatment, apparent nitrogen recoveries (ANR) were calculated as in Louro et al. (2015).

3.2.10. Meteorological data

Daily rainfall and daily mean air temperatures were recorded from 13th April to 20th July 2011 and from 6th October to 21th December 2011 by the weather station located at CIAM. Monthly mean rainfall and air temperatures of the last 10 years (2001-2010) and the corresponding means for these parameters in 2011 were also used.

3.2.11. Statistical analysis

Statistical analyses were performed using Sigmaplot (11.0). Two way repeated measures analysis of variance (ANOVA) was used to determine significant differences between treatments within each period and between periods for the parameters cumulative and mean

cumulative N_2O, CH_4 and CO_2 fluxes and CO_2 equivalents. For soil (WFPS and mineral N contents) and plant (DM yield and N uptake) parameters, the same test was used to investigate significant differences between treatments within sampling days. For yield scaled emission factors (expressed as CO_2 equivalents per DM yield), $EF_{Napplied}$ and $EF_{Nuptake}$ and apparent nitrogen recoveries, one way ANOVA was used to determine significant differences between treatments. Student-Newman-Keuls' test was used to determine which treatment groups were different at the $P \leq 0.05$ significance level.

3.3. Results

3.3.1. Weather conditions

Rainfall in 2011 was 30% lower than the 10 year mean value (2001-2010) (Figure 3.1). Except in April, where the rain was 20% larger compared to the last 10 year mean, rainfall in the rest of months was significantly lower with respect to the same mean. Thus, rainfall records were 23, 37, 29 and 44% lower than average in winter (January-March), spring (April-June), summer (July-September) and autumn (October-December), respectively. Total rainfall was 129 mm during the spring experimental period (13th April-6th June), 19 mm during the period sampled in summer (14th June-20th July) (Figure 3.2a) and 352 mm during the sampling in autumn (6th October-21st December) (Figure 3.2b).

The annual mean air temperature in 2011 was 13.7ºC, 0.5ºC warmer than the last 10-years (2001-2010). Spring and autumn in 2011 were warmer compared to the same mean (Figure 3.1). Mean air temperatures during the spring (13th April-6th June) and summer (14th June-20th July) sampling periods were 14.9 (daily mean temperatures ranged from 11.3 ºC to 18.3) and 17.4 ºC (daily mean temperatures from 14.4 to 23.5 ºC), respectively (Figure 3.2a). During the autumn sampling period (6th October and 21st December), the mean air temperature was 12.1ºC with daily mean air temperatures between 5.9 and 17.7ºC (Figure 3.2b) .

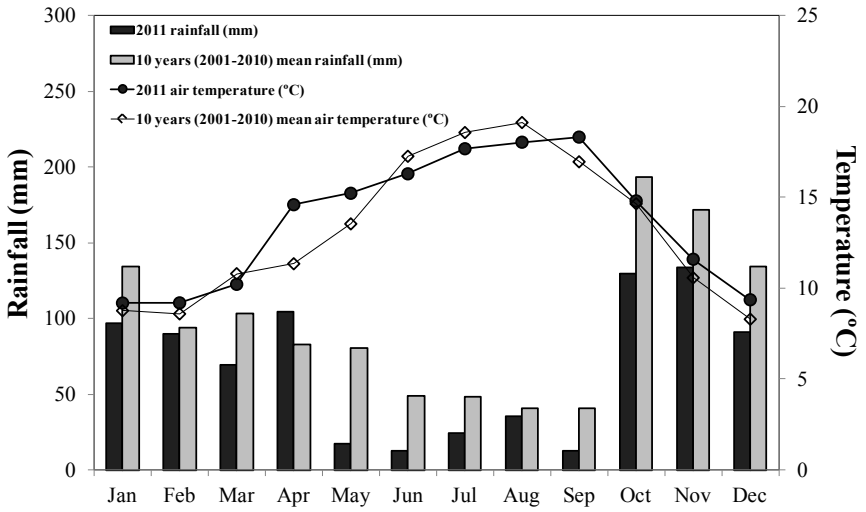

Figure 3.1. Monthly rainfall and mean air temperatures in 2011 and the 10 years average (2001-2010).

3.3.2. Soil WFPS

Soil WFPS in the spring period (13th April-6th June) ranged from 37% to 78%WFPS (Figure 3.2c). In summer (14th June-20th July), these values ranged from 26 to 32% WFPS (Figure 3.2c). In autumn (6th October-21st December), soil WFPS increased from mean contents of 24%WFPS to 81%WFPS (Figure 3.2d). In this period, soil WFPS contents in CS plots were larger than the rest of treatments. However, those contents were only significantly larger on 14th November, six days after the third fertilization (P<0.05).

3.3.3. Soil mineral N

3.3.3.1. Ammonium contents

In spring, fertilization (first fertilization: 13th April) did not significantly increase soil ammonium (NH_4^+-N) contents (0-10 cm) in CS and MN plots, with respect to CN (P<0.05) (Figure 3.3a). Soil NH_4^+-N contents were in the ranges 8.7-21.6, 14.8-23.8, 12.4-33.1 kg N ha^{-1} in CN, MN and CS plots, respectively. In summer, soil NH_4^+-N levels in MN plots were greater than those in CS and CN plots (P<0.05) after the second fertilization (14th June) (Figure 3.3a). During this period NH_4^+-N contents were in the ranges 9.5-43.8 kg N ha^{-1} in CN, 52.2-73.3 kg N ha^{-1} in MN and 16.8-28.8 kg N ha^{-1} in CS. It was observed that soil NH_4^+-N levels largely increased in CN plots during this period, even when no N was added. In autumn, before the third fertilization (8th November), treatments showed soil NH_4^+-N contents in the ranges 16.0-

33.5, 18.3-40.1 and 19.1-60.1 kg N ha^{-1} in CN, CS and MN, respectively (Figure 3.3b). After the third fertilization, these values were in the ranges 8.2-34.5 kg N ha^{-1} in CN, 8.5-48.7 kg N ha^{-1} in MN and 9.3-23.6 kg N ha^{-1} in CS plots. Significant differences were observed between MN and the rest of the treatments on 6th October and on 21st November (P<0.05).

3.3.3.2. Nitrate contents

In spring, soil nitrate (NO_3^--N) contents were in the ranges 5.1-21.6, 14.8-37.1 and 12.8-43.6 kg N ha^{-1} for CN, MN and CS plots, respectively after the first fertilization (Figure 3.3c). In summer, MN plots showed soil NO_3^--N contents in the range 34.3-73.3 kg N ha^{-1} after the second fertilization, which were significantly larger than those in CN (4.9-28.8 kg N ha^{-1}) and CS (3.3-9.5 kg N ha^{-1}) plots (P<0.05) (Figure 3.3c). In autumn, before the third fertilization, soil NO_3^--N levels in MN plots were also significantly higher than the rest of treatments (P<0.05) (Figure 3.3d). The largest soil NO_3^--N levels of the experiment were observed in all treatments on 24th October, fifteen days before the third fertilization. On that day, soil NO_3^--N values in MN plots reached levels of 154.5 kg N ha^{-1}, significantly higher than those of 98.3 and 101.6 kg N ha^{-1} in CS and CN plots, respectively. Immediately after the peak observed on 24th October, soil NO_3^--N contents decreased in the following days prior the third fertilization (Figure 3.3d). After the third fertilization (8th November), soil NO_3^--N contents in MN plots were in the range 18.2-71.2 kg N ha^{-1}, which were significantly larger than the ranges 9.6-17.4 and 12.6-28.4 kg N ha^{-1} in CN and CS plots, respectively (P<0.05).

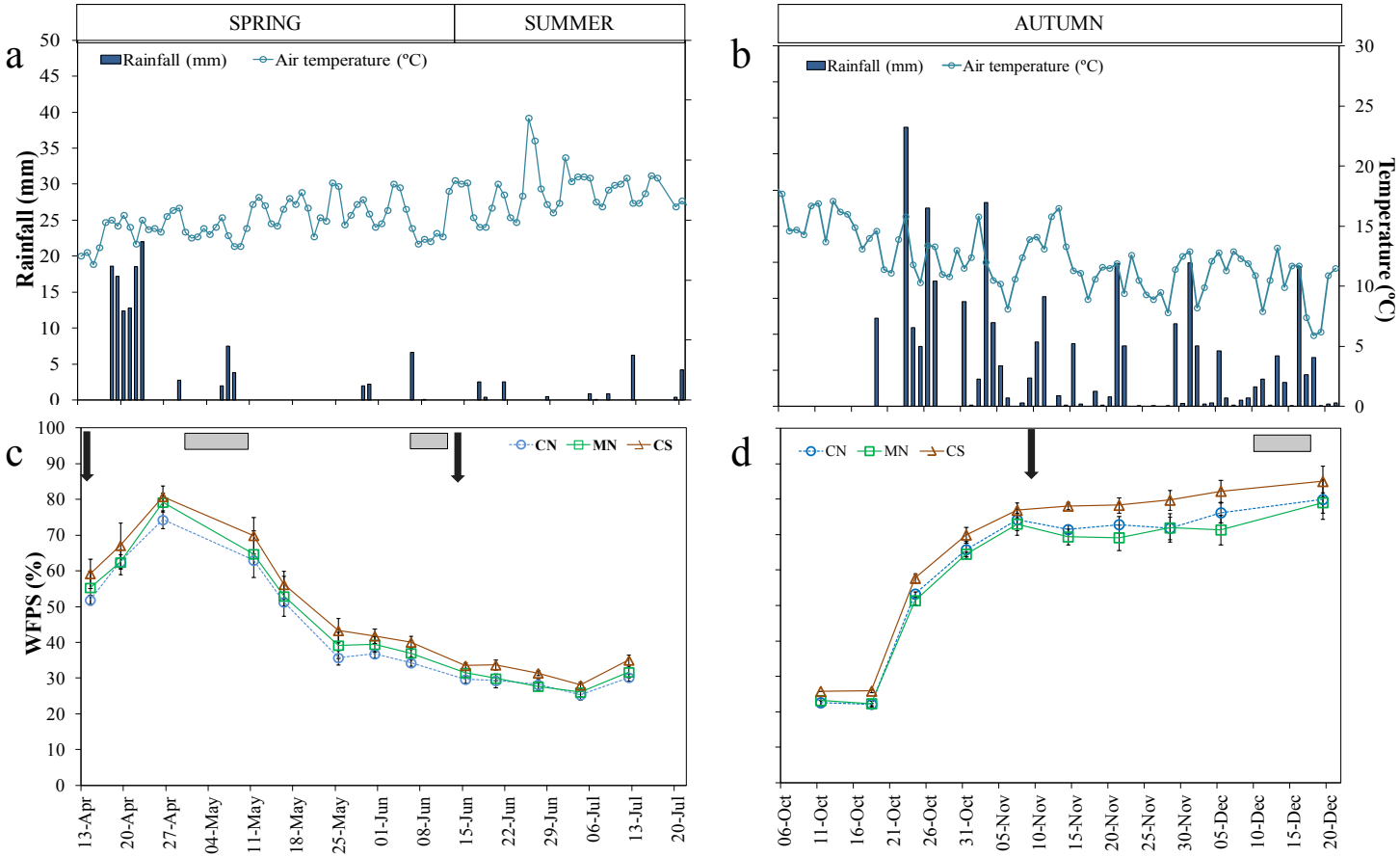

Figure 3.2. Daily rainfall and daily mean air temperatures registered during the sampling periods in (a) spring, summer and (b) autumn. Soil water filled pore space (WFPS) in the samples taken during (c) spring, summer and (d) autumn. In WFPS figures, data represent the mean value of the three replicates and error bars showing the standard error. Arrows represent fertilizations (13th April, 14th June, 8th November) and each grey box represents a grazing period. Treatments: CN: control; MN: mineral fertilizer; CS: injected cattle slurry. Period monitored in spring (13th April-6th June), summer (14th June-20th July) and autumn (6th October-21st December).

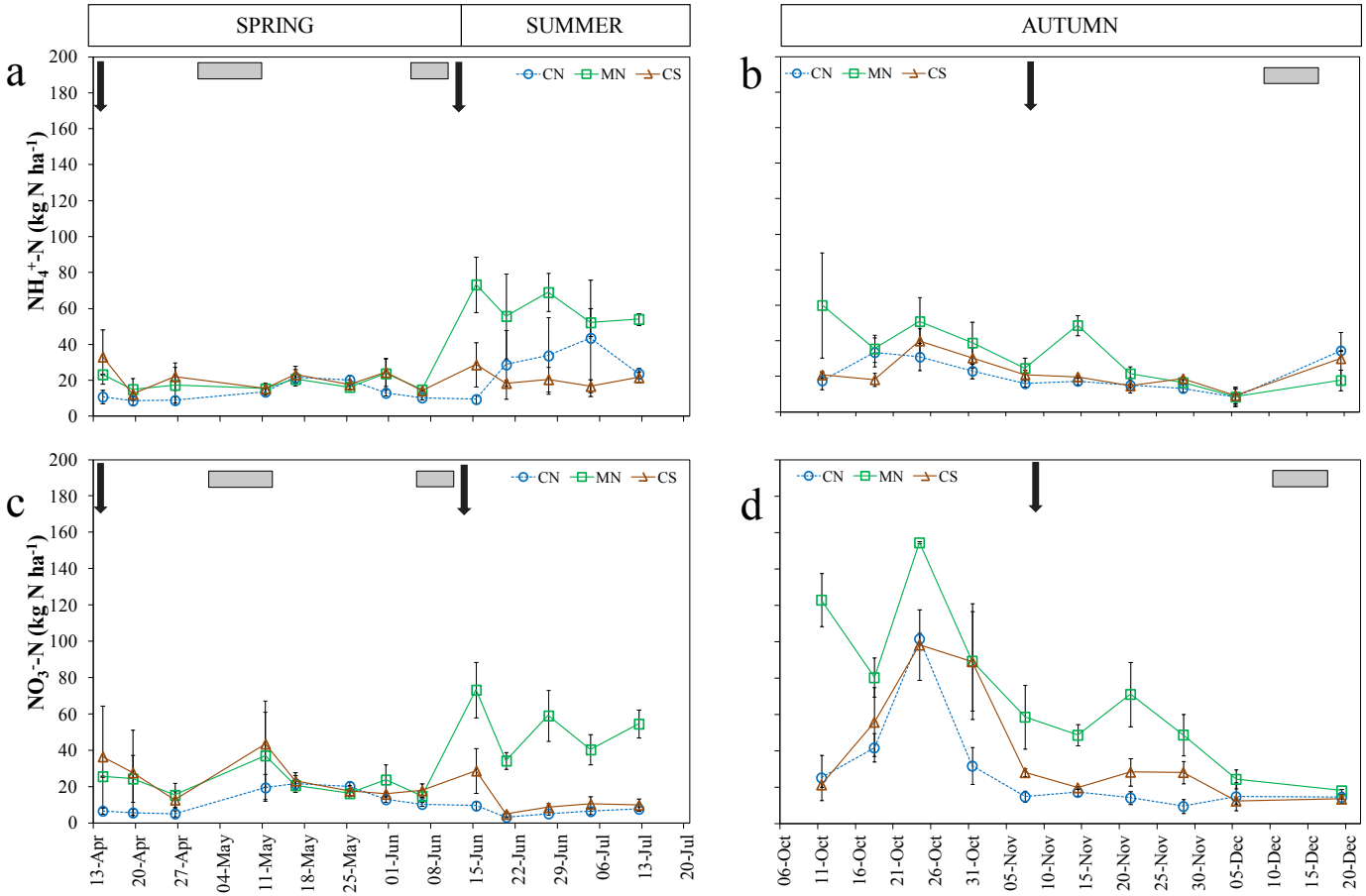

Figure 3.3. Soil NH_4^+-N contents in the samples taken during (a) spring, summer and (b) autumn. Soil NO_3^--N contents in c) spring, summer and d) autumn. Data points represent mean value of the three replicates and error bars, the standard error. Arrows represent fertilizations (13th April, 14th June, 8th November) and each grey box represents a grazing period. Treatments: CN: control; MN: mineral fertilizer; CS: injected cattle slurry. Periods monitored in spring (13th April-6th June), summer (14th June-20th July) and autumn (6th October-21st December).

3.3.4. Greenhouse gas (GHG) flux measurements

Greenhouse gas fluxes were measured at least five times during the first week after fertilization, decreasing the frequency in the following weeks and before the next fertilization (1-3 times a week). A total of 44 gas samplings were carried out during the experiment: 15 during spring (13th April to 6th June), 9 after in summer (14th June-20th July), 20 samplings in autumn (6th October-21st December).

In order to investigate significant differences between treatments caused by fertilization and differences between fertilization themselves, the first 30-36 days after each fertilization were separated from the rest of sampling days and designated as 'A' (first fertilization), 'C' (second fertilization), 'E' (third fertilization). the remaining days after next fertilization or end of the experiment were designated as 'B', 'D' and 'F' (Figure 3.4).

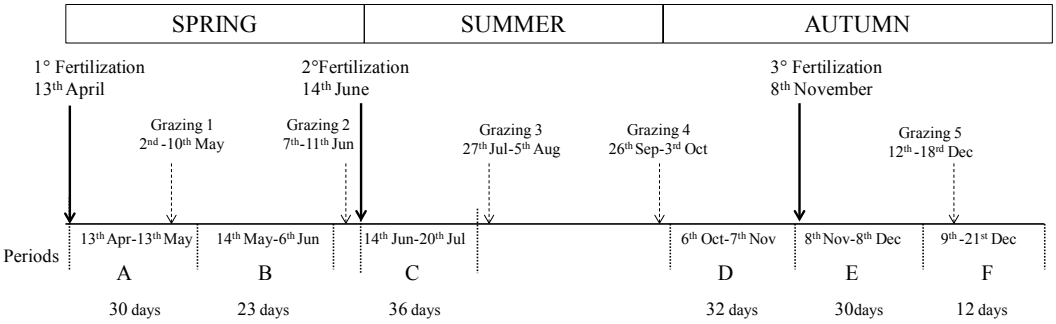

Figure 3.4. A schematic representation of the experiment and GHG fluxes measured during the periods monitored between 13th April and 21st December 2011.

3.3.4.1. Nitrous oxide fluxes

In spring, the first fertilization resulted in N_2O peaks of 0.112 and 0.092 kg N_2O-N ha^{-1} day^{-1} seven days after MN and CS applications, respectively, with their magnitude decreasing to values below 0.02 kg N_2O-N ha day^{-1}in the following days (Figure 3.5a). In summer, the second fertilization did not result in distinct N_2O peaks. Nitrous oxide fluxes ranged from -0.001 to 0.006 kg N_2O-N ha^{-1} day^{-1} from CN, from -0.002 to 0.006 kg N_2O-N ha^{-1} day^{-1} from MN and from 0.000 to 0.008 kg N_2O-N ha^{-1} day^{-1} from CS plots (Figure 3.5a). In autumn, large N_2O fluxes of 0.075, 0.056 and 0.033 kg N_2O-N ha^{-1} day^{-1} from MN, CN and CS plots were observed fifteen days before the third fertilization (Figure 3.5a). After the third fertilization, CS showed a small increase in the N_2O emitted from fluxes lower than 0.010 kg N_2O-N ha^{-1} day^{-1} to 0.026 kg

N_2O-N ha^{-1} day^{-1}. However, the largest N_2O fluxes resulting from fertilization were observed seventeen days after the third application of the treatments, with fluxes of 0.067, 0.053 and 0.046 kg N_2O-N ha^{-1} day^{-1} from CS, MN and CN plots, respectively (Figure 3.5a).

3.3.4.2. Methane fluxes

Methane fluxes from CN and MN plots were negative or low during the experiment (Figure 3.5b). Values ranged from -56 to 39 and from -56 to 38 g CH_4 ha^{-1} day^{-1} from CN and MN plots, respectively. Slurry injection resulted in short-lived and high CH_4 fluxes immediately after applications and on the following day (only after the first and second fertilization), and decreased in the next days to values as from CN and MN plots (Figure 3.5b). Maximum CH_4 fluxes of 462, 365 and 548 g CH_4 ha^{-1} day^{-1} appeared immediately after the first (in spring), second (in summer) and third (in autumn) slurry fertilizations, respectively.

3.3.4.3. Carbon dioxide fluxes

Carbon dioxide fluxes from CN and MN plots were in the ranges 0.9-56.9 and 1.6-58.2 kg CO_2-C ha^{-1} day^{-1}, respectively (Figure 3.5c). Large CO_2 fluxes of 61.1, 67.7 and 58.7 kg CO_2-C ha^{-1} day^{-1} were observed from CS plots immediately after the first, second and third fertilization, respectively. In the rest of the days, CS plots showed similar trend as that observed in MN and CN plots.

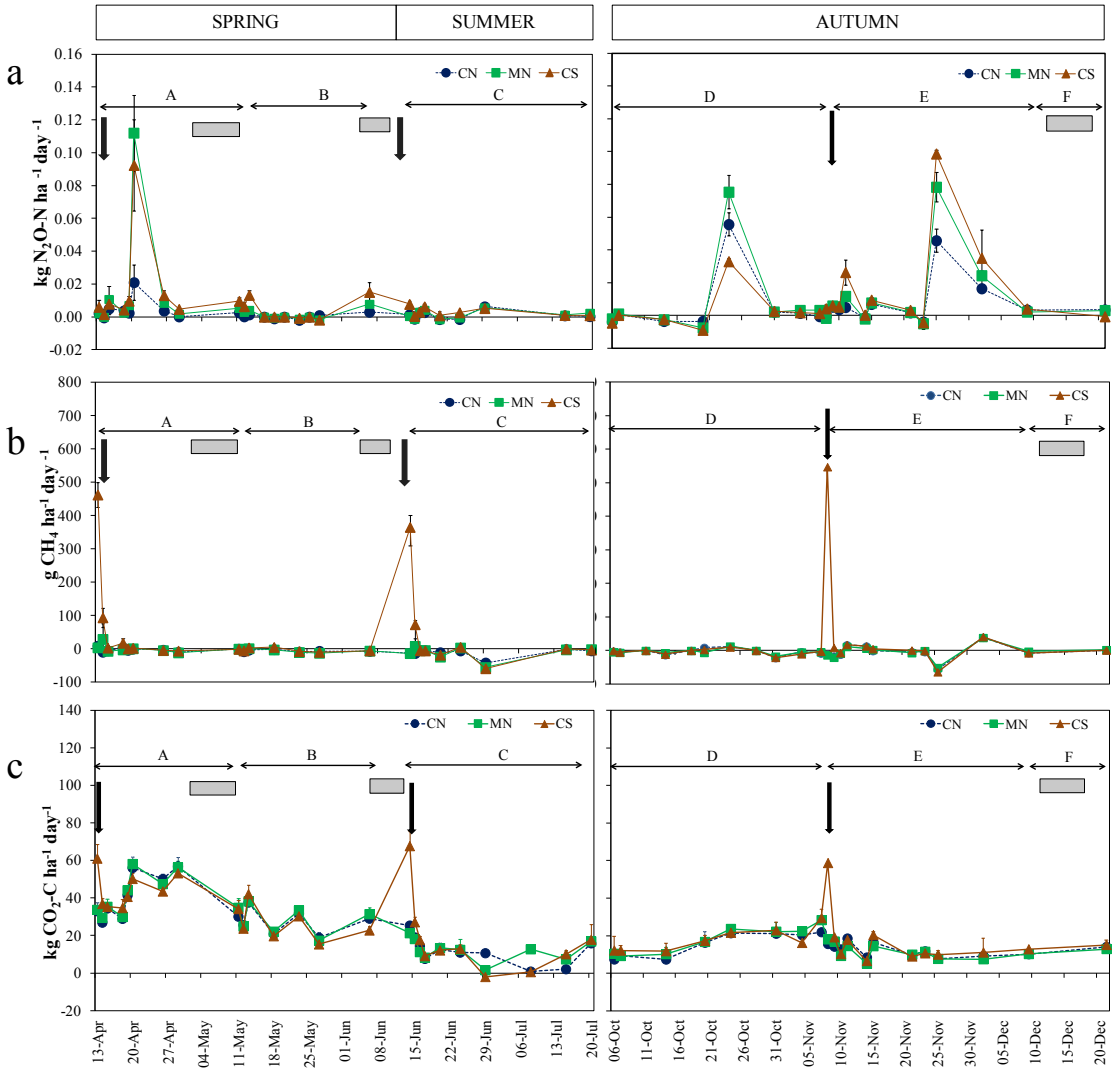

Figure 3.5. Temporal pattern of the a) N_2O, b) CH_4 and c) CO_2 fluxes during the periods measured between 13th April and 21st December. Data represent mean value of three replicates and standard error. Arrows represent fertilizations (13th April, 14th June, 8th November) and each grey box represents a grazing period. Treatments: CN: control; MN: mineral fertilizer; CS: injected cattle slurry. Periods: 'A'- 13th April-13th May; 'B'- 14th May-6th June; 'C'- 14th June-20th July; 'D'- 6th October-7th November; 'E'- 8th November-8th December; 'F'- 9th December-21st December.

3.3.5. Cumulative GHG fluxes

Total cumulative fluxes of N_2O, CO_2 and CH_4 for the whole experimental period (13th April–21st December) and in the periods studied (see Figure 3.4) are shown in Table 3.4. Significant differences between N-treatments (MN and CS) were observed during the periods 'D' and 'E' ($P<0.05$) but overall, total cumulative N_2O fluxes between MN and CS were similar ($P>0.05$) (Table 3.4). In period 'D' mean cumulative N_2O fluxes from MN plots were 2.8-fold larger than from CS ($P<0.05$) (Figure 3.6). The opposite situation was observed in period 'E' where CS increased 1.4-fold mean cumulative N_2O fluxes with respect MN ($P<0.05$). During periods 'A' and 'E', mean cumulative N_2O fluxes from CS and MN plots were the largest ($P<0.05$). Overall, $EF_{Napplied}$ were 0.60% (±0.2) after an annual application of 121 kg N ha^{-1} as mineral fertilizer and 0.28% (±0.6) after the injection of 258 kg N ha^{-1} as cattle slurry (0.50% ±0.11 when only the mineral fraction applied with the slurry is considered) and these differences were not significant ($P>0.05$).

Total cumulative CH_4 fluxes from the CS treatment were significantly greater than the rest of the treatments at the end of the experiment. Cattle slurry injection caused larger cumulative CH_4 fluxes than MN and CN in the periods following fertilizations (i.e. periods 'A', 'C' and 'E') ($P<0.05$) (Table 3.4). Comparing those periods, the largest mean cumulative CH_4 fluxes from CS plots were in period 'E' and the lowest in period 'C' (Figure 3.6). In the periods ('B', 'D' and 'F'), cumulative CH_4 fluxes from CS were similar than MN and CN ($P>0.05$) treatment. In MN and CN plots, the lowest mean cumulative CH_4 fluxes (average -15.7 g CH_4 ha^{-1} day^{-1}) were observed in period 'C' and the largest in period 'E' (0.1 g CH_4 ha^{-1} day^{-1}).

Total cumulative CO_2 fluxes from CS plots were slightly larger than the rest of treatments at the end of the experiment but not significantly different ($P>0.05$). Although, large CO_2 fluxes were observed immediately after CS injection, the resulting cumulative CO_2 fluxes from CS plots were not significantly larger than MN and CN plots neither in the periods immediately after fertilizations (i.e. 'A', 'C', 'E') nor in the rest of periods (P>0.05) (Table 3.4). Comparing periods, the largest mean cumulative CO_2 fluxes from all treatments were observed in period 'A' and the lowest in period 'C' ($P<0.05$) (Figure 3.6).

In terms of total CO_2 equivalents, CS plots resulted in larger CO_2 equivalents than MN and CN plots only during the period 'E' ($P<0.05$) but, overall, there were no significant differences in total CO_2 equivalents caused by the use of N-fertilizers (CS or MN) compared to no fertilization (CN) ($P>0.05$) (Table 3.4). In all treatments, the largest CO_2 equivalents occurred during period 'A' which caused 35-37% of the total CO_2 equivalents of the experiment ($P<0.05$) (Table 3.4).

Table 3.4. Cumulative fluxes of N_2O (kg N_2O-N ha^{-1}), CH_4 (kg CH_4 ha^{-1}) and CO_2 (Mg CO_2-C ha^{-1}) and total CO_2 equivalents (N_2O+CH_4+CO_2, in Mg equiv CO_2-C ha^{-1}) from the different treatments in the different periods studied and for the overall experiment. Mean value of three replicates and standard error (between brackets). Treatments: control (CN); mineral fertilizer (MN); injected cattle slurry (CS). Greenhouse gas fluxes were not measured during the period between 21st July and 5th October. Letters indicate that treatments within each period and gas were statistically different ($P < 0.05$).

		Cumulative GHG emissions						
		Spring		Summer	Autumn			
Gas	Treatment	'A' 13 April-13 May	'B' 14 May-6 June	'C' 14 June-20 July	'D' 06 October-7 November	'E' 8 November-8 December	'F' 9-21 December	Total experiment 13 April- 21 December
N_2O	CN	0.13 (0.06)b	0.03 (0.01)a	0.07 (0.01)a	0.28 (0.05)ab	0.37 (0.06)b	0.04 (0.01)a	0.92 (0.04)b
	MN	0.52 (0.12)a	0.06 (0.05)a	0.08 (0.02)a	0.39 (0.15)a	0.47 (0.06)b	0.03 (0.01)a	1.55 (0.12)a
	CS	0.54 (0.11)a	0.17 (0.06)a	0.11 (0.02)a	0.14 (0.01)b	0.66 (0.04)a	0.02 (0.01)a	1.65 (0.13)a
CH_4	CN	-0.12 (0.09)b	-0.15 (0.01)b	-0.56 (0.10)b	-0.14 (0.06)a	0.00 (0.06)b	-0.04 (0.01)a	-1.01 (0.12)b
	MN	-0.10 (0.10)b	-0.19 (0.06)b	-0.57 (0.13)b	-0.15 (0.02)a	0.01 (0.07)b	0.00 (0.02)a	-1.00 (0.05)b
	CS	0.52 (0.09)a	-0.11 (0.04)b	-0.04 (0.03)a	-0.18 (0.00)a	0.61 (0.25)a	-0.04 (0.03)a	0.78 (0.17)a
CO_2	CN	1.34 (0.08)a	0.80 (0.03)a	0.30 (0.05)a	0.52 (0.01)a	0.35 (0.03)a	0.16 (0.01)a	3.46 (0.14)a
	MN	1.40 (0.07)a	0.83 (0.05)a	0.31 (0.06)a	0.70 (0.13)a	0.31 (0.03)a	0.15 (0.01)a	3.70 (0.19)a
	CS	1.32 (0.10)a	0.90 (0.01)a	0.35 (0.03)a	0.58 (0.03)a	0.39 (0.04)a	0.18 (0.01)a	3.73 (0.11)a
Total CO_2 equivalents	CN	1.40 (0.10)a	0.81 (0.04)a	0.32 (0.05)a	0.65 (0.01)a	0.52 (0.04)b	0.18 (0.01)a	3.87 (0.15)a
	MN	1.65 (0.12)a	0.86 (0.06)a	0.33 (0.06)a	0.88 (0.19)a	0.53 (0.03)b	0.17 (0.01)a	4.41 (0.24)a
	CS	1.59 (0.08)a	0.98 (0.02)a	0.40 (0.04)a	0.64 (0.02)a	0.72 (0.01)a	0.19 (0.01)a	4.52 (0.10)a

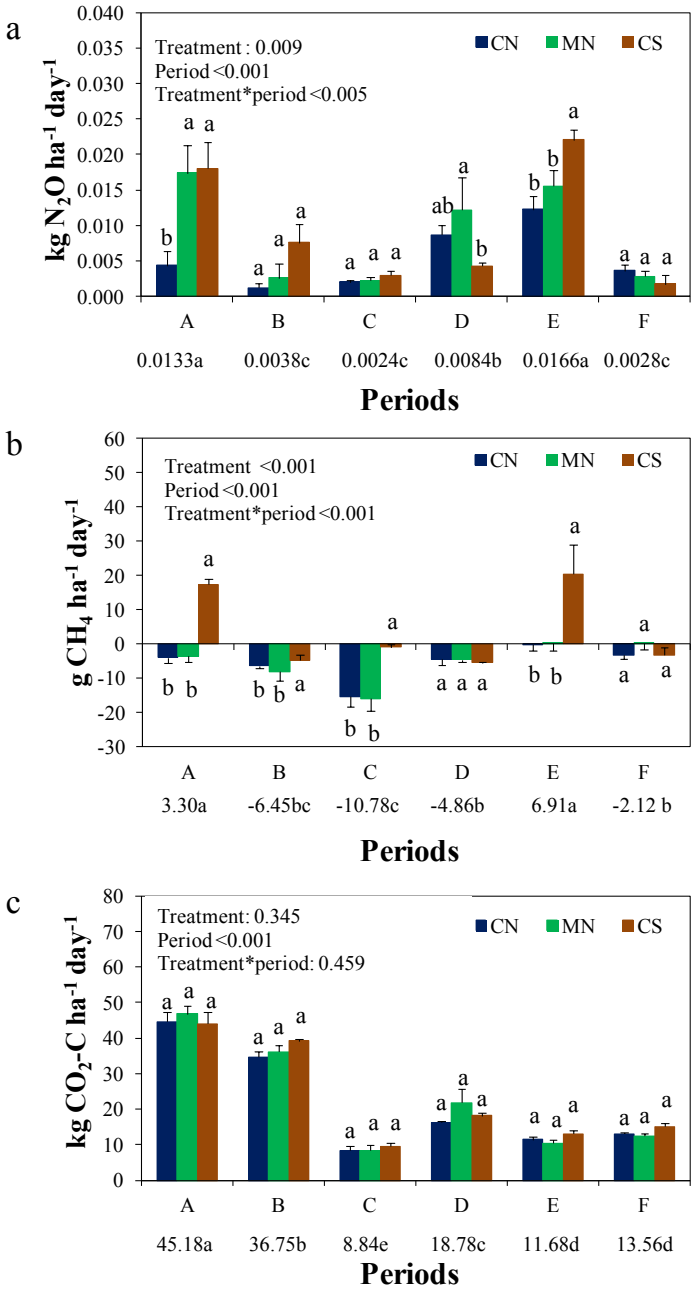

Figure 3.6. Results of a two way ANOVA repeated measures and pairwise multiple comparison test (Student-Newman-Keuls test) for mean cumulative fluxes of (a) N_2O, (b) CH_4 and (c) CO_2. Bars and error bars show the mean and standard error (n = 3). Values below the x-axis show mean value of the periods ('A', 'B', 'C', 'D', 'E', 'F'). Means and bars with different letter show a significant difference using Student-Newman-Keuls test ($P > 0.05$). Treatments: CN: control; MN: mineral fertilizer; CS: injected cattle slurry. Periods: 'A'- 13th April-13th May; 'B'- 14th May-6th June; 'C'- 14th June-20th July; 'D'- 6th October-7th November; 'E'- 8th November-8th December; 'F'- 9th December-21st December.

3.3.6. Grass yields, N uptake and yield scaled emission factors

No significant differences between treatments were observed in the resulting DM yields and plant N uptake in any grazing event (P>0.05) (Table 3.5). The largest DM values and plant N uptake were obtained in the grass sampling 1 (prior to grazing event 1) and the lowest in the grass sampling 4 (prior to the fourth grazing event) (P<0.05). In terms of emission factors ($EF_{Nuptake}$), N-fertilizer treatments were almost 1.5-fold larger than CN (P<0.05). Overall, total CO_2 equivalents per crop yield (Table 3.5), from MN and CS plots were slightly higher but not significant different than CN (P>0.05). Apparent nitrogen recovery (ANR) from MN plots were slightly higher than CS but not significant different (P>0.05) than CS, either when ANR from MN is compared with the mineral N fraction or total N contained applied with CS. These ANR were 41%±11% in MN and 10%±10 and 17%±17 from CS when total N and mineral N fractions are considered, respectively.

Table 3.5. Dry matter yield (Mg ha^{-1}), N uptake (kg N ha^{-1}) resulting from the samples taken prior to each grazing event. Emission factors based on the resulting yields (Mg equiv CO_2-C Mg DM^{-1}) and based on N uptake by plant (%). Mean values of the three replicates and the standard error (between brackets) are presented. Treatments: control (CN); mineral fertilizer (MN); injected cattle slurry (CS). Yield scaled emission factors do not include the period between 21st July and 5th October as GHG fluxes were not measured. For each parameter, treatments within each column with different letter were statistically different (P < 0.05). Grass samplings: 2nd May (sampling 1); 9th June (sampling 2); 26th July (sampling 3); 21st September (sampling 4); 12th December (sampling 5).

		Grass sampling 1	Grass sampling 2	Grass sampling 3	Grass sampling 4	Grass sampling 5	Total	Yield scaled emission factor
Parameter		Mg DM ha^{-1}						Mg equiv CO_2-C Mg DM^{-1}
DM								
	CN	4.53 (0.38)a	1.67 (0.03)a	1.55 (0.07)a	0.49 (0.04)a	1.51 (0.10)a	9.75 (0.45)a	0.40 (0.02)a
	MN	4.75 (0.06)a	1.87 (0.21)a	1.64 (0.18)a	0.47 (0.04)a	1.77 (0.29)a	10.50 (0.38)a	0.42 (0.04)a
	CS	4.67 (0.38)a	1.43 (0.18)a	1.32 (0.04)a	0.57 (0.13)a	1.74 (0.28)a	9.74 (0.73)a	0.47 (0.03)a
N uptake		Kg N ha^{-1}						$EF_{Nuptake}$ (%)
	CN	96 (16)a	37 (2)a	20 (5)a	10 (1)a	51 (3)a	220 (14)a	0.42 (0.01)b
	MN	122 (4)a	40 (3)a	33 (2)a	10 (0)a	63 (9)a	270 (9)a	0.58 (0.05)a
	CS	113 (3)a	30 (4)a	25 (2)a	13 (3)a	60 (9)a	245 (11)a	0.67 (0.03)a

3.4. Discussion

3.4.1. Nitrous oxide fluxes

Large ranges in annual N_2O losses have been reported from other European grasslands under grazing and fertilization management and similar climatic conditions as in NW Spain (Burchill et al., 2014; Cardenas et al., 2010; Hyde et al., 2006; Rafique et al., 2012; Rafique et al., 2011; Rees et al., 2013). The resulting N_2O losses, between 0.5 and 51.3 kg N ha^{-1}, are caused by the large difference in the N management (annual N inputs of fertilizer between 100 and approximately 525 kg N ha^{-1} plus N inputs from livestock grazing) in combination with the large variability in the soil sites characteristics and climatic conditions (836 to 1183 mm). The resulting N_2O losses from this 7-month experiment was within the range of values observed from those European grasslands. However, according to a previous study carried out between 2007-2009 in grazed grasslands with mineral N fertilizer applications at CIAM-INGACAL (Chapter 2), the losses obtained in this study were significantly lower. According to the earlier study annual N_2O losses vary from 8.8 to 30.1 kg N_2O ha^{-1}, with the largest losses observed in wet years in combination larger annual grazing intensities. In this experiment, gas samplings were carried out under atypical climatic conditions, which was reflected in $EF_{Napplied}$ values from both types of fertilizer, which were below the IPCC default value of 1% (IPCC, 2006). As it was mentioned, in comparison with the last 10-years mean for this region, 2011 was a dry and warm year. All seasons where gas sampling were carried out had a considerable reduction in the rainfall patterns in comparison with the last 10-years mean. However, the reduction seriously affected spring and summer seasons as rainfall was very low.

3.4.1.1. Nitrous oxide fluxes during spring and summer periods

According to the earlier study (reported in Chapter 2), spring and summers periods are large contributors to the annual N_2O emissions, as the high frequency of rainfall is combined with large N inputs from fertilizers and livestock excretion, resulting in large losses. In this experiment, as rainfall was low and air temperatures were very warm during spring and summer, low soil WFPS levels (<60%WFPS) were frequently observed, causing a general trend of low N_2O fluxes throughout most of the sampling days in these periods. In the period after first application (period 'A'), the only N_2O peaks from N-fertilized treatments were observed when fertilization was followed by a short period of days with high rainfall (Dobbie and Smith, 2003; Smith et al., 2012), significantly increasing (although for a short-term) soil WFPS (up to

78%WFPS). Under these soil conditions, denitrification could cause these short-lived losses of N_2O seven days after the first fertilization, changing the general pattern of low soil N_2O fluxes, which were probably caused by nitrification (Bateman and Baggs, 2005).

These short-term soil WFPS increases were not observed after the second fertilization (period 'C'). Because of the little rain and the increase of air temperatures in this period, the soil reached extremely low WFPS levels (30%WFPS, mean value, Figure 3.2c), which resulted in even lower N_2O fluxes compared to the previous period. With the soil aerobic conditions in this period only nitrification could have been responsible for the N_2O fluxes observed. However, no nitrification of the N applied with fertilizers was observed during this period. That was very clear in CS plots where mineral N was applied entirely as NH_4^+-N (Table 3.1) and no increase in the soil NO_3^--N was observed in the following days after fertilization (Figure 3.3c). We also observed that OM mineralization would have been stimulated during the previous periods (as an increase in mineral N was observed in CN plots), providing additional NH_4^+-N to the soil (Figure 3.3a). However, as in CS plots, soil NO_3^--N contents did not increase (Figure 3.3c). In this sense, microbial population could be affected by the low soil moisture levels in combination with warm temperatures, reducing the activity and causing the low N_2O fluxes observed. That could explain the large fraction of mineral N accumulated in the soil at the end of the period 'C'.

3.4.1.2. Nitrous oxide fluxes during the autumn periods

The mineral N accumulation in the soil from previous fertilizations and the N depositions during the summer grazing events could be the N pool for the large N_2O losses observed in the period prior to third fertilization (period 'D'). In this period, the frequent rain increased significantly soil WFPS (up to 60%WFPS) compared to in summer. That would have activated the microbial population, resulting in the high N_2O fluxes observed from the N- fertilizer treatments and also from CN plots. A similar situation has been reported by Cardenas et al. (2010), Kim et al. (2010) and Rafique et al. (2012) from grasslands in UK and Ireland. Kim et al. (2010) and Rafique et al. (2012), in Ireland, attributed the large N_2O pulses, when rainfall preceded by long dry periods, to nitrification. They observed that N_2O fluxes at soil WFPS below 60% were better correlated to soil NH_4^+-N contents rather than NO_3^--N. In this study, N_2O production would have been caused by denitrification even when the fluxes were observed at 54% WFPS (mean value, Figure 3.2d). This was based on the fact that those peaks coincided with large peak of soil NO_3^--N in all treatments (Figure 3.3d) which decreased immediately after the N_2O peaks. After the period of soil rewetting, the frequency of rain was larger and soil remained in a constant anaerobic soil status (WFPS>60%), optimal conditions for large losses

by denitrification. That explains the two episodes of N_2O fluxes observed from CS and MN after the third fertilization (period 'E'). In this period, denitrification would have been more stimulated in CS plots than in MN. The reason could have been in the more anaerobic conditions in CS, probably because of the slightly larger soil WFPS contents in CS plots (Figure 3.2d) caused by the water contained in the slurry. This could explain the differences observed after the third fertilization (period 'E') between N-treatments.

3.4.2. Methane fluxes

In soils, CH_4 fluxes are the net result of CH_4 production by methanogenesis (positive fluxes) and CH_4 oxidation (negative fluxes) by methanotrophy processes (Baggs et al., 2006; Ball et al., 1999). In this experiment, the net result from CS plots was CH_4 production whereas in MN and CN plot the net result was CH_4 oxidation of approximately 1 kg CH_4 ha^{-1}. The reason of the result obtained from CS plots is attributed to large fluxes observed immediately after each cattle slurry application, which are frequently observed where manures are applied to soil and as it was observed in this experiment, the origin of those fluxes would come from the release of the dissolved CH_4, generated in the slurry, but not from the soil (Chadwick and Pain, 1997; Flessa and Beese, 2000; Rodhe et al., 2006; Sherlock et al., 2002). One proof of the non-soil origin of those large fluxes immediately after CS applications is that they were observed within the first day after application and they rapidly decreased s in the following days to reach similar magnitudes as in MN and CN plots. This is because methanogenesis is sensitive to O_2 and diffusion of O_2 into the cattle slurry from the soil inhibits CH_4 formation (Chadwick et al., 2011). If the fluxes at the time of CS applications in 'A', 'C' and 'E' were excluded in all treatments (data not shown), CS plots showed similar cumulative CH_4 fluxes as for CN and MN treatments, denoting that methanotrophy was the dominate process during the experiment.

Comparing the resulting CH_4 fluxes following CS applications, it was observed that the injection in autumn produced greater CH_4 fluxes than in spring and summer. A possible explanation could be the lower DM contents of the material applied during the third fertilization, which could aid infiltration into the soil (Chadwick and Pain, 1997), promoting more anaerobic conditions in the soil for methanogenic processes. However, the slurry applied in summer contained similar DM contents as that used in autumn. In agreement with Chadwick et al. (2000) and Louro et al. (2013), the largest CH_4 fluxes observed in this experiment appear to be also greater under wetter and cooler climatic conditions and the reason could be attributed to the fact CH_4 oxidizers would have delay CH_4 consumption due to the more anaerobic conditions in autumn, thus increasing the rate of CH_4 released (Rodhe et al., 2006). This is also

supported by the seasonal trend in CH_4 consumption observed in CN and MN plots. Atmospheric CH_4 consumption followed an increasing trend with decreasing soil WFPS and increasing air temperatures (affecting finally soil temperatures) which is in agreement with the observations reported by Louro et al. (2013) and Meijide et al. (2010). When mean cumulative CH_4 fluxes from MN and CN plots in the different periods are compared, the largest mean CH_4 oxidation rates of this experiment coincided with the lowest soil WFPS levels and the largest air temperatures of the experiment observed during period 'C' (Figure 3.6b), contrasting with the lowest mean CH_4 oxidation rates during autumn where climatic conditions were the opposite.

Some studies have shown that the N fertilizers can inhibit CH_4 oxidation due to the competition between the NH_4^+-N and CH_4 for the methane monooxigenase, the enzyme responsible for the oxidation of CH_4 and others highlight the importance of additions of N for nitrifying population, which also oxidises methane (Bodelier and Laanbroek, 2004). In addition, other studies report that the inhibitory effects in the CH_4 oxidation capacity of the soil is caused by the to the toxic effect of high concentrations of NO_3^--N, which is likely give rise to osmotic potential (Hütsch et al., 1996; Nesbit and Breitenbeck, 1992). In this experiment, no inhibition of caused by N fertilizer additions was observed. The largest mean CH_4 oxidation rates in soils treated with slurry and mineral fertilizer occurred under significant levels of NH_4^+-N (in CS and MN plots) and NO_3^--N (in MN plots) and no differences were observed with the corresponding CH_4 oxidation rates from CN plots. A possible explanation of this result could be in the large fertility of the soil (Dobbie and Smith, 1996), reason why the addition of N fertilizers did not have an immediate effect in the resulting oxidation rates.

3.4.3. Carbon dioxide fluxes

Many studies have reported that grasslands switched from a net carbon sink in a wet and normal year to a net source in drought conditions (Aires et al., 2008; Du et al., 2012; Meyers, 2001; Nakano and Shinoda, 2014). As it was mentioned, the year 2011 does not correspond to a normal year (according to the 10-year average) as reductions in rainfall were observed in all seasons. As result, the grassland resulted in a source of CO_2, which is consistent with the findings in the studies cited above, under drought conditions.

Carbon dioxide fluxes obtained with the chamber methodology represent ecosystem respiration. Ecosystem respiration is influence by disparate factors, those that affect heterotrophic and autotrophic respiration. Heterotrophic respiration has been reported to be strongly regulated by soil moisture and temperature (Davidson et al., 2000; Xu and Qi, 2001) and autotrophic respiration varies seasonally as the relative roles of growth and maintenance respiration change

(Falge et al., 2002). In this sense, according to Davidson et al. (2000), heterotrophic respiration has a linear increase with temperature but also requires moisture conditions near field capacity where macropore spaces are mostly air-filled, which facilites O_2 diffusion, and micropore spaces are mostly water-filled, to facilitate diffusion of soluble substrates. Based on this, the largest ecosystem respiration observed during the spring period in this grassland soil would be explained by the coincidence of high plant growth peak, in addition to warm temperatures and optimal moisture soil conditions for high heterotrophic respiration. Meijide et al. (2010) observed that ecosystem respiration increased in autumn when soil was fallow and it was rewetted after the lack of rainfall during summer, resulting in pulses of CO_2. In this experiment, the soil rewetting episode in autumn (period 'D') resulted in large N_2O fluxes from all treatments but clear CO_2 pulses were not observed. Instead, we observed that cumulative CO_2 fluxes in period 'D' were twice as large as in period 'C'. The only large fluxes observed in this experiment were those related to cattle slurry applications, which is consistent with other studies after organic fertilizer applications to the soil (Fangueiro et al., 2008b; Flessa and Beese, 2000; Meijide et al., 2010; Rochette et al., 2004). According to some studies the cause of the CO_2 flux observed after slurry applications may be attributed to the release of the CO_2 dissolved in the slurry which is released after the application to the soil or even CO_2 production from the dissociation of slurry carbonates (Fangueiro et al., 2008b; Flessa and Beese, 2000; Rochette et al., 2004). Rochette et al. (2004) reported an absence of CO_2 fluxes immediately after slurry injection in autumn and large fluxes after the spring application. They hypothesised that some CO_2 could be solubilised into the soil solution under high soil water contents and low temperatures in autumn. In this experiment, although the differences in the resulting CO_2 fluxes following each slurry injection were not very large (range 58.7-67.7 kg CO_2-C ha^{-1} day^{-1}), the lowest CO_2 flux was in autumn, coinciding with the highest soil WFPS values and the lowest temperatures, and the largest flux, in summer, when conditions were the opposite. In this sense, similarity in CO_2 fluxes would have been the result of the net effect of the different slurry carbonate contents between the slurries and the different soil conditions at the moment of each fertilization. Despite these fluxes, ecosystem respiration from CS were not different than MN or CN plots. As CS resulted in similar yields as those obtained in CN and MN plots after each application, the fraction of autotrophic respiration would have been similar among treatments, denoting that slurry applications to this soil would not change microbial activity. The reason of this result would have been caused by the large amount of organic C present in this soil (48 g kg^{-1}) as consequence of the grazing history in this site. Under these conditions, slurry-C additions could not have caused a larger CO_2 response because microbial population would have obtained enough substrate from the soil.

3.4.4. Nitrogen uptake and yield scale emission factors

As it was mentioned, the dry soil conditions during spring and summer could have stimulated mineralization of the organic matter and that was reflected in the large N uptake obtained from CN plots. The large supply of N from organic matter mineralization caused the low efficiency of the fertilizers applied, suggesting that large proportion of the N applied with fertilizers was not taken up by grass, leaving part of the N in the soil, in agreement with Schils et al. (2008). The resulting ANR from both types of fertilization were not significant different. However, the corresponding the fraction recovered from AN were slightly higher than the recovery obtained with CS. This difference could be attributed to some losses of the ammoniacal fraction of the slurry by ammonia volatilization caused by the shallow injection technique (1-25% of the total ammoniacal N, Huijsmans et al. (2001)). As consequence of possible large contribution of the OM mineralization and due to the low N_2O losses observed during the experiment, the $EF_{Nuptake}$ in this experiment derived from N-fertilizers were much higher than range of 0.2-0.3% reported in other European grasslands soils with only N inputs from fertilizer applications (Hansen et al., 2014; Schils et al., 2008) but much lower than the resulting values of up to 8% from grasslands soils with annual N inputs from grazing (up to 203 kg N ha^{-1}) and fertilization (up to 156 kg N ha^{-1}) (resulting from data provided in Burchill et al. (2014)), under more wetter soil conditions.
The aim of this study was to select the type of fertilizer type related to low GHG emissions and large grass yields. We observed that both fertilizers caused similar losses of total CO_2 equivalents to produce same yields. Based on this, both fertilizers could be used. However, if the costs of purchasing mineral fertilizers are considered, using injected slurries as fertilizer would be more beneficial for dairy farmers as animal wastes produced on farms would be recycled and milk production costs would be reduced.

3.5. Conclusion

Dry weather conditions during the spring and summer limited N_2O production after fertilizations and caused a general trend of low and similar N_2O losses after the application or mineral fertilizer or cattle slurry. Only, in spring ,when fertilization was followed by a period of rain, short-lived N_2O losses were observed when fertilization was followed by a period of rain. Simultaneously, the dry weather conditions during these periods would have stimulated organic matter mineralization, providing quantities of N that met crop demands. That resulted in large part of the added N left in the soil, which was lost in early autumn by denitrification when soil was rewetted. Under the wetter conditions in autumn, application of cattle slurries caused larger losses of N_2O than MN probably because of the larger soil WFPS levels. Large losses of CH_4

and CO_2 were related to slurry injection but overall only total cumulative CH_4 fluxes were significant respect to the mineral fertilizer. The reason of the differences in total CH_4 were caused by the large release of the CH_4 contained in the slurry after each application but not from the soil. The aim of this study was to find the type of fertilizer associated with low GHG fluxes and large yields in a grazed grassland. We observed that both fertilizers caused similar total CO_2 equivalents to produce same yields. Based on this, both fertilizers could be used. However, if the costs of purchasing mineral fertilizers is considered, using injected slurries as fertilizer would be more beneficial for dairy farmers as animal wastes produced on farms would be recycled and milk production costs would be reduced.

CHAPTER 4. EFFECT OF SLURRY AND AMMONIUM NITRATE APPLICATION ON GREENHOUSE GAS FLUXES OF A GRASSLAND SOIL UNDER ATYPICAL SOUTH WEST ENGLAND WEATHER CONDITIONS

Louro, A., Sawamoto, T., Chadwick, D., Pezzolla, D., Bol, R., Baez, D., Cardenas, L., 2013. Effect of slurry and ammonium nitrate application on greenhouse gas fluxes of a grassland soil under atypical South West England weather conditions. Agric. Ecosyst. Environ. 181, 1-11. DOI:10.1016/j.agee.2013.09.005

Abstract

In this study we evaluated how typical split applications of cattle slurry (SL) or mineral fertilizer (AN) (in spring, summer and autumn) affected greenhouse gas (GHG) emissions from a grassland soil. Field measurements were carried out between May and November in 2011 using the closed chamber technique. The experiment was located in the South West of England, an area which is typically characterized by high annual precipitation (>1000 mm yr^{-1}) and cool temperatures (average annual air temperature of 9.6^{o}C). The unusual dry climatic conditions observed during the late spring and summer, and the rainfall events identified in autumn affected soil water filled pore space (WFPS) resulting in low nitrous oxide (N_2O) fluxes during the experiment. After the first two applications, climatic conditions dried the soil to values below 60%WFPS, the threshold level for losses of N_2O by nitrification. In contrast, the frequent rainfall events observed after the third application (in autumn) increased the WFPS and promoted losses of N_2O by denitrification. In terms of fertilizer type, AN resulted in higher cumulative N_2O emissions compared with SL after the third application, probably because the SL treatment resulted in more anaerobic soil conditions and ammonia (NH_3) volatilisation resulted in a smaller mineral N pool in the soil available for N_2O production and emission. Ammonia (NH_3) emission modelling estimated losses of N by volatilization of NH_3 between 25-38% of N applied after slurry surface broadcast application. Plant N offtake represented nearly all of the total N applied in AN plots following the first two applications and 59% of that applied in the third, whereas in SL plots an average of 64% of the total N applied in the three applications was harvested in the grass. Nitrogen gas (N_2) fluxes were not measured but the large rainfall events observed after the third application gradually increased the soil WFPS to saturation and could also have resulted in losses of N by complete denitrification, especially from the AN treatment. Thus, applications of AN and SL resulted in total N_2O-N losses during the 6-month measurement period of 0.21 and 0.17 kg N ha^{-1}, respectively (representing only 0.02 and 0.003% of the N applied). Methane (CH_4) production was observed in the first two or three days after SL spreading. For the remaining days, and also in plots treated with AN, the soil acted as a sink of CH_4 (consumption). Total net CH_4 cumulative values of -0.09 and 0.92 kg CH_4 ha^{-1} were observed in AN and SL, respectively. CH_4 consumption and production rates were related to changes in the %WFPS. Thus, dry soil conditions (below 60%WFPS) enhanced the CH_4 consumption, observed in May and June in AN, and reduced the rate of CH_4 production in SL plots. Total net cumulative carbon dioxide (CO_2) fluxes of 1.24 and 0.35 Mg CO_2-C ha^{-1} were observed in AN and SL plots during the 6-months measurements.

4.1. Introduction

Agriculture and agricultural production practices play an important role in the global fluxes of the greenhouse gases (GHG) methane (CH_4), nitrous oxide (N_2O) and carbon dioxide (CO_2), each contributing 6.5%, 5.5% and 0.1% to the total anthropogenic greenhouse gas emissions, respectively (Sänger et al., 2011). The production or consumption of these gases are mainly due to biological processes which are strongly affected by natural conditions and agricultural management (Snyder et al., 2009). Weather conditions and the addition of slurries or mineral fertilizers (such as ammonium nitrate (NH_4NO_3)) can significantly affect the interchange of greenhouse gases between the soil and the atmosphere. This is due to an increase in the supply of substrates and a change in soil conditions that alters the processes responsible of the production and/or consumption of each gas. Nitrification and/or denitrification can produce N_2O emissions. Both processes occur if nitrogen (N) is applied in the ammonium (NH_4^+-N) form, but only by denitrification if it is applied solely in the nitrate (NO_3^--N) form (Clayton et al., 1997) or after NH_4^+-N has been converted to NO_3^--N. Nitrification is responsible for the release of N_2O in dry or well-aerated soils (as it is an aerobic process), while denitrification is, dominantly, the main pathway at medium-high soil water contents (being an anaerobic processes). The production of CH_4 is primarily by microbial degradation of organic matter under anaerobic conditions, e.g. in wetlands or from rice paddies. However, the application of animal manures can provide an immediately available carbon source and moisture leading to CH_4 production (Chadwick and Pain, 1997). Well-aerated upland soils (e.g. grasslands, forest and arable) are commonly regarded as a biological sink of atmospheric CH_4 and are responsible for 6% of the global methane consumption (Le Mer and Rogert, 2001). Carbon dioxide (CO_2) emissions resulting from respiration in soil and vegetation are the principal sources of CO_2 entering the atmosphere (Smith et al., 2003). Applications of livestock slurries and mineral fertilizers provide nutrients for plant and soil microbial growth, and enhance CO_2 emissions, although N fertilizer effects on soil CO_2 emissions are dependent on weather conditions during the growing season (Morell et al., 2011). Soil moisture influences gas exchange by altering oxygen availability and gas diffusivity. Soil respiration has been shown to increase with increasing temperature due to the enhanced microbial activity. Because of varying weather and soil conditions, the GHG fluxes are likely to be temporally dynamic and also dependent on the nature of fertilizer inputs.

The main aim of this study was to investigate how typical management of slurry or mineral fertilizer affect GHG emissions in a grassland soil in the South West of England. We applied cattle slurry and NH_4NO_3 to grassland plots on three occasions over a period of six months. The fluxes of N_2O, CH_4 and CO_2 were measured at high frequency using the static chamber

methodology. Although unplanned, the experiment was carried out under atypical, i.e. unusually dry, climatic conditions for the region.

4.2. Material and methods

4.2.1. Location

The field trial was carried out in permanent grassland in May 2011 at Rothamsted Research, North Wyke, Devon, UK (50:46:10N, 3:54:05W), which has a temperate maritime climate (Koppen, 1931), typical of the South-West England. The 40-year mean annual temperature (1961-2000) is 9.6ºC, and the minimum and maximum monthly mean temperatures are 4.5ºC in February and 15.5ºC in August. The mean annual precipitation (40-years average) is 1056 mm, 46% of which falls between October and January. British soil classification (Avery, 1980) defines the soil as clayey typical non-calcareous pelosol of the Halstow series and as a stagni-vertic cambisol, and as aeric haplaquept using FAO and USDA taxonomy, respectively. The soil texture was a silty clay loam (Harrod and Hogan, 1981). Initial analysis of the properties of the upper 10 cm of the soil profile indicated a total N content of 0.44%, total carbon (C) content of 3.94%, C:N ratio of 8.9, pH of 5.9 and bulk density (BD) of 0.88 Mg m^{-3}.

4.2.2. Experimental design

The experiment was set up in a randomised block design, with three replicate plots of the following treatments: (1) control with no N application (zero N), (2) mineral fertilizer as NH_4NO_3 (AN) and (3) cattle slurry (SL). Replicate plots were 15.0 m^2 in area (3.0 m x 5.0 m), and within each main replicate plot three zones were marked out separating chamber, soil sampling and grass yield measurement areas. Within each plot, three chambers were fixed within an area of 3.0 m^2 (3.0 m x 1.0 m), whilst two zones each of 6.0 m^2 in area (3.0 m x 2.0 m) were left for the soil sampling and grass yield measurements. A distance of 1 m was kept between blocks and treatments.

4.2.3. Application events and characteristics of mineral fertilizer and slurry used

The applied NH_4NO_3 comprised 34.5% N. Cattle slurry was collected from a dairy farm located near North Wyke. Treatments were applied by hand three times during the experiment (1st application: 16th May, 2nd application: 24th June and 3rd application: 8th September) at a target rate of 80 kg N ha^{-1} (8 g N m^{-2}) on each application. Slurry was analyzed for total N prior to each application (Table 4.1). Slurry was spread at rates of 2.26 l m^{-2} in May and June and 2.48 l m^{-2} in September, and the NH_4NO_3 at the rates of 23.2 g m^{-2}, equivalent to 80 kg total N ha^{-1} at each of the three applications.

4.2.4. Chamber design and operation

The closed chamber technique was used to quantify the GHG flux measurements in the field (Rochette and Erisksen-Hamel, 2008). Chambers comprised white polyvinyl chloride (PVC) open ended boxes with a volume of 0.032 m^3 (length 40 cm, width 40 cm, height 30 cm, Cardenas et al. (2010)). The upper edge of the chamber had a 'U' shaped channel, which was filled with water to ensure an airtight seal with the PVC lid. The lid was fitted with a sampling port with a three-way valve. Three chambers were used per replicate plot (i.e., 27 chambers in total). To ensure a good seal between the chamber and soil, the chambers were inserted into the soil to a depth of 10 cm > 24 h before the flux measurements began (Parkin and Venterea, 2010), and left in the same place until the first plot harvest to avoid soil disturbances, which could affect the soil-atmosphere gas transfer. Chambers were returned to the same position after each plot harvest. The effective height of each chamber above the ground (H) was measured three times: at the beginning of experiment (5th May) and after the first and second grass harvests (22nd June and 24th August). Height measurements were taken internally at the centre of each wall and in the centre of the chamber. The resultant chamber effective height was the mean of the 5 points taken, and ranged between 20.6-25.4 cm. Values recorded were used to calculate GHG fluxes from each chamber after the insertion in the soil. On each sampling occasion, the chamber was closed for 40 min. Ten ambient air samples were taken (5 at the start of the chamber closure, and 5 at the end of the 40 minute period) to provide background values for N_2O, and stored in pre-evacuated 20 ml glass vials. The average of N_2O, CH_4 and CO_2 concentrations in these ambient air samples was used as the time zero sample (T0). After 40 minutes (T40) the chamber headspace was sampled via the three-way valve on the chamber lid. 60 ml gas samples were taken from each chamber headspace (and from ambient air) and transferred to 20 ml pre-evacuated glass vials, using a syringe and hypodermic needle to inject

the sample into the vial. A second needle was used to release the sample to ambient pressure. Soil surface temperature inside chambers was also recorded every sampling date and the data used to calculate the gas fluxes.

We assumed a linear accumulation of headspace GHG concentrations in our flux calculations; an observation we have verified for N_2O using the same chambers on the same soil type following AN applications (Cardenas et al., 2010), thus using our resources to ensure we accounted for spatial variability of fluxes within each plot (using three chambers per plot), and opting to sample at high frequency (see next section), improving the ability to account for temporal variability of fluxes. In addition, data collected from a nearby experiment carried out at the same time as the current experiment, showed that CO_2 accumulation in the headspace was linear at 40 minutes in 88% of the chambers tested (Cardenas, L.; Chadwick, D., personal communication).

4.2.5. Greenhouse gas flux measurements and laboratory analysis

Measurements of N_2O, CO_2 and CH_4 were conducted frequently over a 6-month period, from the 16th May to 13th November 2011. Gas sampling was usually carried out between 10:00 and 12:00 h. Chambers were sampled every day during the first fifteen days after AN and SL applications, and every two days until the next application. Gas samples were usually analyzed within 24 h of collection with a Perkin Elmer Auto-system gas chromatograph (GC500) equipped with two Elite Plot Q columns (30 m x 0.53 mm) and two detectors: ^{63}Ni electron capture detector (ECD) at 300ºC for measuring N_2O and a flame ionization detector (FID) at 350ºC for CO_2 equipped with a methanizer to analyze CH_4. This system was attached to an auto-sampler (Perkin Elmer headspace sampler Turbo matrix 110), which extracted a sample of 0.03µl/min from the sampling vial and injected it into the GC. Calibrations were performed using standards of N_2O, CO_2 and CH_4 (0.33, 1.59, 5.23 ppm for N_2O; 2.97, 1198, 2467 ppm for CO_2; 2.05, 5.18, 10.18 ppm for CH_4). Concentrations of gases were calculated by comparing peak areas integrated with those obtained with the standards of each gas.

Fluxes were calculated from the accumulation of gas in the chamber as follows (Eq.1):

$$F = \rho \times H \times (C_{40}-C_0)/ t \times 273.15/ T \times 24 \times 10^4 \quad \text{(Eq.1)}$$

where F is the gas flux (kg N_2O-N ha^{-1} day^{-1}, g CH_4 ha^{-1} day^{-1}, kg CO_2-C ha^{-1} day^{-1}), ρ is the gas density (N_2O-N: 1.26 kg m^{-3}; CH_4: 717 g m^{-3}; CO_2-C: 0.536 kg m^{-3}) under STP conditions

(273.15 K, 101,325 Pa), H is the effective height of the chamber (m), C_{40}-C_0 is the gas concentration at 40 minutes (T40) after chamber closure minus gas concentration of ambient sample (T0) ($\mu m^3\ m^{-3}$), t is the time of chamber closure (40 min) and T the temperature (K) inside the chamber. Cumulative emissions were calculated by the trapezoidal method (Cardenas et al., 2010; van den Pol-van Dasselaar and Oenema, 1997; Velthof et al., 1996).

For N_2O and CH_4, emissions from the zero N treatment were subtracted from the corresponding values in fertilized plots for the whole period of measurements, giving net emissions attributable to the N applied (for N_2O) or to the amount of slurry (for CH_4). Seasonal N_2O emission factors (EFs), for each amendment studied during the experiment, were calculated by dividing the net N_2O cumulative fluxes by the total N applied and expressing as percentage. Methane emission factors, expressed as %C applied, were calculated for each application of slurry by dividing the net cumulative CH_4 fluxes by the total amount of slurry applied.

4.2.6. Ammonia (NH_3) losses

Although NH_3 losses were not measured in the field, an estimation of the percentage of NH_3 loss from each application was calculated using the empirical model, ALFAM (Ammonia losses from Field-Applied Animal Manure), provided by Søgaard et al. (2002). The ALFAM model is a multiple regression model based on empirical data of NH_3 loss from experiments conducted in seven European countries (UK, Denmark, the Netherlands, Norway, Sweden, Switzerland, and Italy) and it uses the Michaelis-Mentel type equation to predict NH_3 loss over time (t) after slurry application as:

$$N(t) = N_{max} \times (t/(t+K_m)) \quad \text{(Eq.2)}$$

where $N(t)$ is the cumulative loss fraction of total amoniacal nitrogen (TAN), N_{max} (as % NH_4^+-N applied) the total time integrated loss and Km the time (h) in which 50% of NH_3 loss occurred. The instantaneous emission rate corresponds to the derivated dN/dt of Eq. (2) as follows:

$$dN/dt: N_{max} \times (K_m \times (t+K_m)^{-2} \quad \text{(Eq.3)}$$

For the estimation, variables related to slurry, (type of slurry (pig/cattle), application technique, DM and NH_4^+-N contents), weather (air temperature, wind speed) and soil conditions (dry/wet) are required model inputs because these significantly affect $Nmax$ and Km.

In this experiment, NH_3 losses were estimated to provide an understanding of a major loss pathway of available N, which could be used to help explain the patterns of N_2O emissions observed. The value of *Nmax* provided after each of the three slurry applications was considered as the cumulative NH_3 loss and expressed as percentage of NH_4^+-N applied with the slurry. Losses of NH_3 from AN applications were estimated using the emission factor of 1.4% (percentage of loss of NH_4^+-N applied) reported by Misselbrook et al. (2004b), in grasslands fertilized with NH_4NO_3 and without grazing management.

4.2.7. Soil sampling and analysis

During the experiment, flux measurements were accompanied by measurements of soil moisture at a depth of 0-10 cm. Every sampling day, one soil core per replicate of each treatment was taken and then unified into one sample per treatment for analysis of gravimetric soil moisture. This parameter was measured by oven-drying the samples at 105ºC for 24 h. Soil BD of the 0-10cm layer was determined at the start of the experiment from five undisturbed blocks of soil with a mean height of 8.6 cm. Measures of the width and length of each side of block were recorded. The volume of soil was calculated and the soil was dried at 105ºC to constant weight to determine the dry weight. Bulk density was finally calculated from the weight of the dry soil and the volume it occupied and expressed as Mg m^{-3}. Particle density (PD) was assumed to be 2.65 Mg m^{-3}. Porosity was calculated according to Eq. (4):

$$Porosity = 1 - (BD/PD) \quad \text{(Eq.4)}$$

Gravimetric soil water content was then converted to % soil water filled pore space (WFPS) using the following equation (Eq.5):

$$WFPS = ((Water\ content \times BD)/ Porosity) \times 100 \quad \text{(Eq.5)}$$

Soil ammonium (NH_4^+-N) and nitrate (NO_3^--N) contents were analyzed once a week. Five cores (0-10cm) per plot were taken from the soil sampling zone in each plot, extracted with 2M KCl (Searle, 1984) for 1 h at 200 rpm in a horizontal shaker (1:2 fresh soil:extractant ratio) and then filtered through Whatman No 5 paper. Extracts were analyzed for NO_3^--N and NH_4^+-N soil contents using a SKALAR SAN ^{Plus} analyser (provided with a SKALAR 5000-02 analyser unit,

a SKALAR 28503902_0 photometric detector, a SKALAR 1050d auto-sampler and a circulating water bath).

4.2.8. Slurry analysis

Slurry samples were analyzed for total N by Kjeldahl (AOAC, 1990). Slurry density was calculated on the basis of the volume occupied by a mass of sample. The pH of the slurry was measured directly with a pH meter (HANNA HI 9025). Total C was also analyzed using a TOC analyzer (Skalar FormacsHT, Breda, The Netherlands). Dry matter (DM) was determined after 24 h of drying at 105ºC. Organic matter (OM) was estimated by loss on ignition (LOI); oven-dried samples (105 ºC) were weighed and then ashed for 24h at 550ºC in a muffle furnace. LOI was calculated as follows (Eq.6):

LOI (%) = ((oven-dried sample weight – ashed sample weight) / oven-dried sample weight) x 100 (Eq.6)

Mineral nitrogen (NO_3^--N and NH_4^+-N) was extracted using 2M KCl. Slurry extracts were analyzed using a SKALAR SAN ^{Plus} analyser (described in soil sampling and analysis section). Analysis of other macronutrients (P, K, Ca, Mg, S) were carried out by a commercial laboratory using the aqua-regia method (ISO, 1995) followed by determination by inductively coupled plasma optical emissions spectrometry (ICP-OES). Chemical and physical properties of the cattle slurry applied to the soil are summarized in Table 4.1.

Table 4.1. Chemical and physical properties of the cattle slurry used on each application.

		Applications		
Properties	Units	1st (16th May)	2nd (24th June)	3rd (8th September)
Density	kg l^{-1}	0.995	1.021	1.006
pH	-	6.9	6.8	7.3
Dry matter	%	8.8	6.4	6.5
Total C	% DM[a]	37.6	39.4	38.4
Total N	% FWt[b]	0.36	0.35	0.32
Total N	% DM	2.58	2.67	2.67
NH_4-N	% FWt	0.16	0.12	0.12
NO_3-N	% FWt	0.00	0.00	0.00
LOI	% DM	74.8	77.7	77.1
Ash	% DM	25.2	22.3	22.9
P as P_2O_5	g l^{-1} FWt	1.11	1.05	1.03
K as K_2O	g l^{-1} FWt	2.99	2.82	2.89
Ca as CaO	g l^{-1} FWt	5.45	3.10	2.37
Mg as MgO	g l^{-1} FWt	0.72	0.74	0.74
S as SO_3	g l^{-1} FWt	0.86	0.79	0.79

[a]DM: dry matter; [b]FWt: fresh weight

4.2.9. Grass yield and composition

All plots were harvested three times, 21th June, 24th August and 31st October, using a reciprocating mower to cut an area of 2 x 1 m^2, at a cutting height of 5 cm. Fresh grass samples were taken from the harvesting zone for analysis for DM. This parameter was determined after 16 h drying at a temperature of 80ºC. Dried and ground grass samples were also analyzed for total N. Both parameters were used to determine N plant offtake after each cut.

4.2.10. Meteorological data

Total daily rainfall, daily average air and soil (10 cm depth) temperatures were recorded between 22nd June and 13th November by a weather station within 500 m of the experimental area. Also, the average wind speed (at 10 m height) for the day of the slurry addition and the following day was used as a model input to estimate NH_3 losses by the ALFAM model, after each application. Average monthly rainfall, air and soil temperature data for the last 40 years (1961-2000) were used to compare the weather conditions observed during the experiment.

4.2.11. Statistical analysis

Statistical analyses were performed using Sigmaplot (11.0). One way ANOVA was used to study the effect of fertilization with NH_4NO_3 or cattle slurry on cumulative GHG emissions following each fertilization event, and for the total experiment. Two way repeated measures ANOVA was used to compare the same effect on mean N_2O, CH_4 and CO_2 fluxes under the different weather conditions and to determine significance of changes in soil (mineral N and WFPS) and grass yields (DM, %N contents, N offtake) during the experiment. Daily fluxes of N_2O, CH_4 and CO_2 were related to corresponding soil and weather parameters using Pearson correlation analysis. The "r" value provided by Sigmaplot was used to prove the influence of these environmental conditions on the pattern of GHGs.

4.3. Results

4.3.1. Weather conditions

The annual mean air temperature in 2011 was on average 0.9 ºC higher than the 40-year mean value (Figure 4.1a). Compared to the 40-year average (1961-2000), 2011 was characterized by warm temperatures in spring (March-May) and autumn (September-November), and relatively cool conditions during summer (June-August). The mean daily air temperature ranged during the sampling period (from 16th May to 13th November 2011) ranged from 6.4 ºC (6th November) to 18.2 ºC (2nd October) (Figure 4.1b). November was the coldest month of the experimental period with a mean monthly temperature of 10.3 ºC, whereas maximum mean monthly temperature was recorded in July (14.5 ºC).

Soil temperature ranged from 9.1 ºC (7th November) to 19.3 ºC (28th July). Daily soil temperature was around 14 ºC in the period after the first N-fertilization and increased to mean values of 16.5 ºC in the period after the second application, coinciding with the summer growing season. Mean soil temperature decreased to 13.3ºC, after the third N application, which coincided with the arrival of the autumn.

Rainfall in 2011 was low compared with the 40-year mean (21% less rainfall) (see Figure 4.1a), particularly in the months of March, April and May (before the first N application) when the precipitation was only 73, 71 and 52% of the 40-year average. Total precipitation during the sampling period was 427 mm, (76, 166 and 186 mm fell during the period after the first, second and third N-fertilization event, respectively) with October as the wettest month and May as the driest. Two substantial precipitation events (more than 20 mm day^{-1}) were recorded on 28th August and on 24th October (Figure 4.1b).

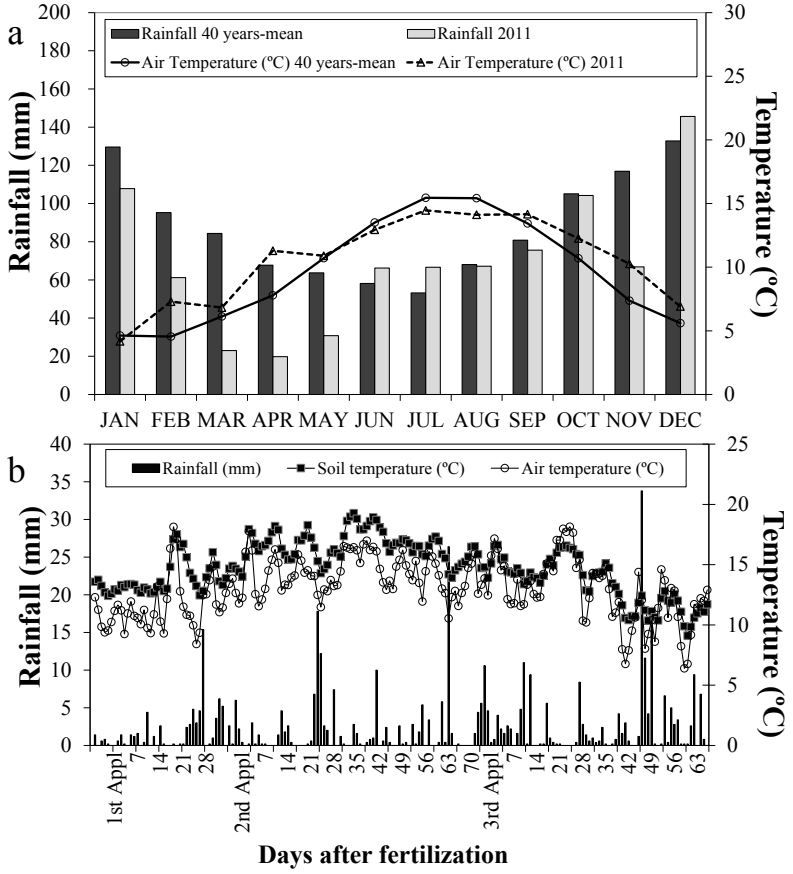

Figure 4.1. (a) Mean monthly rainfall and air temperature in 2011 and the 40-year averages (1961 – 2000). (b) Total daily rainfall and average daily air and soil temperatures recorded during the experiment.

4.3.2. Soil mineral N

After each of the three fertilizer applications in the experiment, only high soil NH_4^+-N values were found in the AN plots (Figure 4.2a), with maximum values of 20.6, 11.0 and 14.8 kg N ha^{-1} in the first, second and third application, respectively. Values of between 0.2 and 2.5 kg N ha^{-1} were found in the zero N plots, and between 0.1 and 3.6 kg N ha^{-1} in the SL plots. Also the highest soil NO_3^--N contents were found in AN plots (Figure 4.2b), with maximum values of 22.4, 15.4 and 17.0 kg N ha^{-1} after the first, second and third N-fertilization, respectively. Zero or very low values of NO_3^--N were recorded in the zero N and SL plots, with values in the range of 0.0-1.4 kg N ha^{-1} from zero N plots and 0.0-1.3 kg N ha^{-1} in SL plots. Two way repeated measures ANOVA showed statistical differences between treatments in NH_4^+-N and NO_3^--N contents with date of sampling (P<0.001) and these contents were significantly higher in AN plots within the first three weeks after each application event. No statistical differences were found between treatments in the soil mineral N contents after this time (for each N-fertilization) (P>0.05).

Based on the significant statistical increases in soil NH_4^+-N and NO_3^--N concentrations during the first three weeks after each fertilization event, GHG emissions data recorded in this experiment were collated into two periods within each fertilization event (Figure 4.3). The period corresponding to the first three weeks of measurements (24 days) after each N application was designated as period 'A', and the remaining days until the next fertilization or end of the experiment, designated as period 'B'.

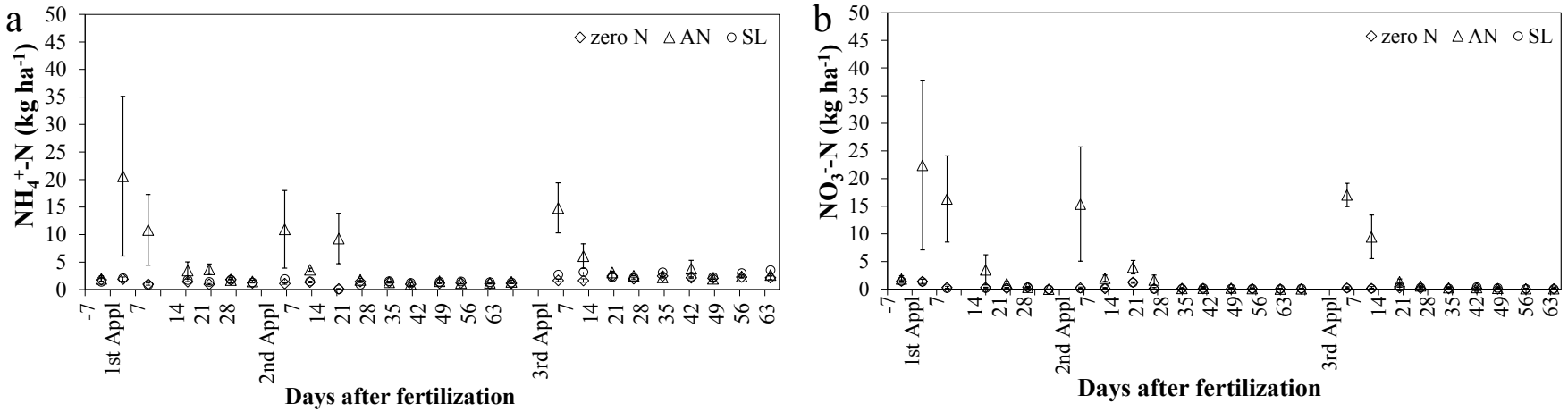

Figure 4.2. Soil inorganic NH_4^+-N (a) and NO_3^--N (b) contents in the fertilized and control treatments during the 6-month study. Treatments: (zero N) unfertilized or control; (AN) ammonium nitrate; (SL): slurry. Each point represents the mean value of three replicates ± standard error.

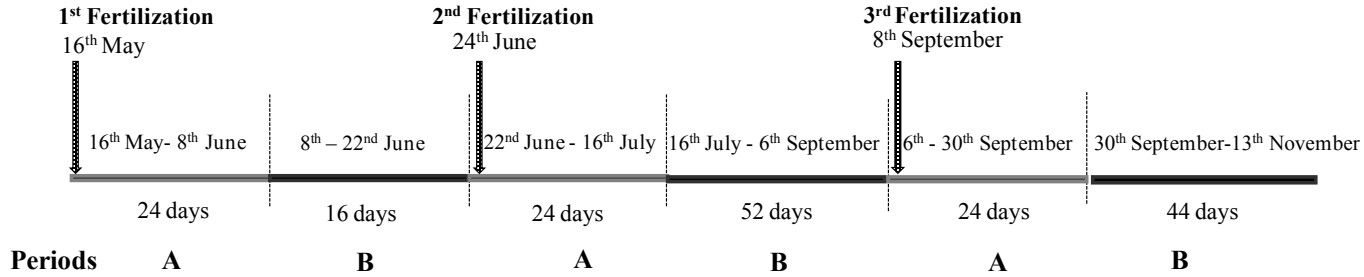

Figure 4.3. A schematic representation of the time periods studied between N applications. The two groups of periods are represented by the letters 'A' and 'B'. Arrows represent the time of fertilization.

4.3.3. Soil WFPS

Low %WFPS values were recorded in the period after the first two fertilization dates (Figure 4.4a). The dry conditions during the spring and summer resulted in WFPS values ranging from 35 to 65%, and 37 to 67% after the first and second application, respectively. These values were below the threshold range, 60-70% WFPS, where denitrification becomes dominant and high N_2O fluxes are often observed (Bateman and Baggs, 2005; Davidson, 1993; Dobbie et al., 1999; Linn and Doran, 1984). After the third application, there was an increased frequency of rainfall events so %WFPS increased to values ranging from 54 to 93% WFPS, which were significantly greater than the WFPS following the first two fertilisation occasions (P<0.001). Statistical analysis showed that the mean soil WFPS value during the latter phase after the autumn application (period 'B') was the highest of the experiment (P<0.001) (Figure 4.5a). However, there was no significant difference in the values of %WFPS of soil samples from zero N, AN and SL plots within each sampling date (P >0.05) and within each period (P>0.05).

4.3.4. Greenhouse gas fluxes

A total of 112 GHG flux samplings were carried out over the 6-month period (16th May to 13th November 2011); 25 after the first N-fertilizer application and 43 and 44 after the second and third applications, respectively. Fluxes of N_2O were low throughout the whole experiment in all treatments studied (Figure 4.4b). Zero N plots resulted in values from -0.004 to 0.006 kg N_2O-N ha^{-1} day^{-1}. Fluxes from AN and SL plots ranged from -0.005 to 0.008 kg N_2O-N ha^{-1} day^{-1} and from -0.004 to 0.004 kg N_2O-N ha^{-1} day^{-1}, respectively. The applications of AN and SL were not followed by a distinct peak of emission following the first application in May. The highest value was observed 15 days after the application of AN and SL and after a period of some rainfall events <5 mm. After the second application, increases in N_2O fluxes were observed on the fourth (higher in AN plots) and twenty fourth days (after amendment applications). After the third application, only AN plots showed an increase in N_2O fluxes from the 10th day to the 14th day, during which time maximum N_2O fluxes were recorded. Forty six days after the third application of the treatments small peaks of N_2O in zero N and SL plots were observed. This continued for 2 further days from the AN plots only.

Fluxes of CH_4 (Figure 4.4c) were generally negative or close to zero from the zero N plots and those fertilized with AN. Values ranged between -11.5 to 6.4 g CH_4 ha^{-1} day^{-1} and between -10.8 to 4.1 g CH_4 ha^{-1} day^{-1} from the zero N and AN treatments, respectively. Only plots fertilized with SL showed distinct peaks of CH_4 fluxes between days 1 and 3 following each slurry

application. Values ranged from -9.8 to 304.5 g CH_4 ha^{-1} day^{-1}. Maximum CH_4 fluxes appeared immediately after each SL application, with values of 65.4 g CH_4 ha^{-1} day^{-1}, 129.6 g CH_4 ha^{-1} day^{-1} and 304.5 g CH_4 ha^{-1} day^{-1} in May, June and September, respectively.

Fluxes of CO_2 (Figure 4.4d) ranged from 5.7 to 91.8, from 8.3 to 115.6 and from 9.9 to 97.4 kg CO_2-C ha^{-1} day^{-1} from the zero N, AN and SL treatments, respectively. Immediately after grass was harvested, CO_2 fluxes were low. Maximum CO_2 peaks in the whole experiment were observed after the first N application.

4.3.5. Cumulative GHG fluxes

Total cumulative fluxes of N_2O, CO_2 and CH_4 for the whole experimental period (16th May-13th November) and in the periods between each fertilizer application and the next fertilization or end of the experiment (1st fertilization: 'A' (16th May-8th June) + 'B' (8th June-22nd June); 2nd fertilization: 'A' (22nd June-16th July) + 'B' (16th July-6th September); 3rd fertilization: 'A' (6th September-30th September) + 'B' (30th September-13th November) are shown in Table 4.2. Also, mean cumulative N_2O, CH_4, and CO_2 fluxes (Figures 4.5b, c and d, respectively) were explored statistically for the periods 'A' and 'B' after each fertilization (see previous definition of these periods).

Total N_2O cumulative fluxes from the AN plots resulted in slightly higher fluxes than those recorded from the zero N and SL treatments, but these differences were not statistically different (P>0.05). Plots fertilized with AN showed significant differences in mean cumulative N_2O fluxes (P<0.05) in the period 'A' after the 3rd N-fertilization compared with those fluxes from the zero N and SL plots (Figure 4.5b). Mean N_2O fluxes from AN plots were 44% and 53% higher than those from SL and zero N, respectively. In terms of N_2O EFs, the total application of 240 kg N ha^{-1} with AN and SL caused a total N_2O-N loss during 6-months measurements of 0.21 and 0.17 kg N ha^{-1}, respectively, accounting for 0.02 and 0.003% of the N applied.

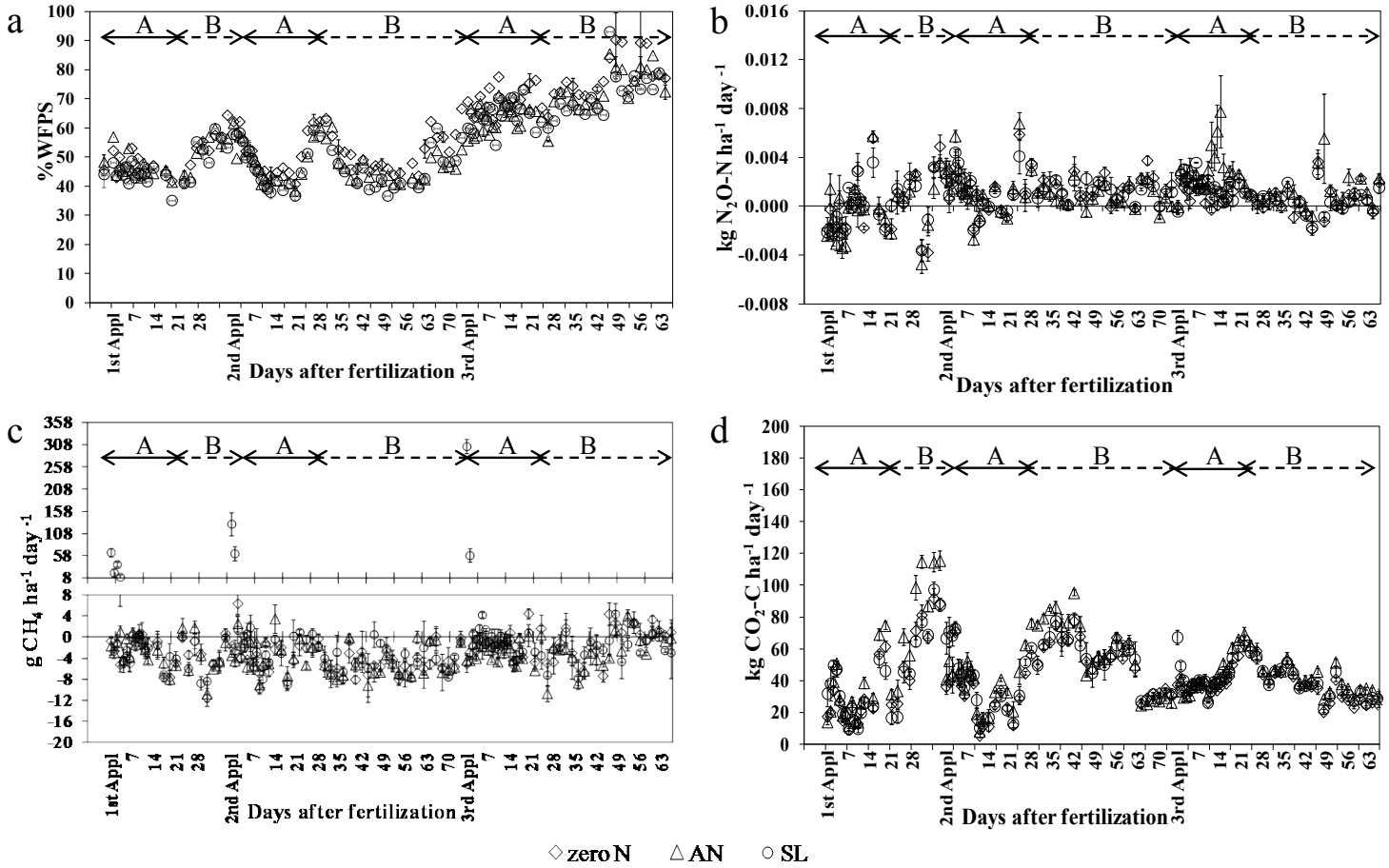

Figure 4.4. (a) % Water filled pore space (WFPS) and daily fluxes of N_2O (b), CO_2 (c) and CH_4 (d). Letters 'A' and 'B' represent the different periods after fertilization and horizontal arrows, their length. Periods - 1st Fertilization: 'A' (16th May-8th June) and 'B' (8th-22nd June); 2nd Fertilization: 'A' (22nd June- 16th July) and 'B' (16th July-6th September); 3rd Fertilization: 'A' (6th-30th September) and 'B' (30th September-13th November). Treatments: (zero N) control; (AN) ammonium nitrate; (SL) slurry. Each point represents the mean value of three replicates ± standard error.

Table 4. 2.Cumulative fluxes after the three fertilizations and total cumulative fluxes of N_2O (kg N_2O-N ha^{-1}), CO_2 (Mg CO_2-C ha^{-1}) and CH_4 (kg CH_4 ha^{-1}). Mean value of three replicates and standard error (between brackets). Treatments: (zero N) control; (AN) ammonium nitrate; (SL) slurry. Fertilizations: 1st: 'A' (16th May-8th June) + 'B' (8th June-22nd June); 2nd: 'A' (22nd June-16th July) + 'B' (16th July-6th September); 3rd: 'A' (6th September-30th September) + 'B' (30th September-13th November); total (16th May-13th November). Treatments with different letter within the same row are statistically different (P<0.05).

		Treatment		
Gas	Fertilizations	zero N	AN	SL
N_2O	1st	0.007 (0.009) a	0.006 (0.003) a	0.007 (0.007) a
	2nd	0.105 (0.026) a	0.089 (0.008) a	0.102 (0.020) a
	3rd	0.048 (0.010) b	0.115 (0.022) a	0.058 (0.006) b
	Total	0.160 (0.045) a	0.205 (0.031) a	0.166 (0.019) a
CO_2	1st	1.59 (0.09) a	2.04 (0.02) a	1.56 (0.14) a
	2nd	3.42 (0.09) a	3.91 (0.16) a	3.60 (0.34) a
	3rd	2.61 (0.04) a	2.90 (0.16) a	2.79 (0.12) a
	Total	7.61 (0.02) a	8.85 (0.22) a	7.96 (0.59) a
CH_4	1st	-0.13 (0.01) a	-0.12 (0.01) a	-0.02 (0.02) a
	2nd	-0.31 (0.03) b	-0.32 (0.02) b	0.01 (0.08) a
	3rd	-0.08 (0.02) b	-0.17 (0.04) b	0.42 (0.01) a
	Total	-0.52 (0.02) b	-0.60 (0.03) b	0.41 (0.10) a

Statistically significant differences were found in total CH_4 cumulative fluxes (Table 4.2) between treatments (P<0.001). Plots fertilized with slurry resulted in fluxes of 0.41 kg CH_4 ha^{-1}, with negative fluxes from the other treatments (-0.52 and -0.60 kg CH_4 ha^{-1}, from zero N and AN plots, respectively). Mean cumulative CH_4 emissions from SL were significantly greater (P<0.001) compared with zero N and AN treatments in the periods 'A' (Figure 4.5c). The quantity of CH_4 emitted in period 'A' increased with successive SL applications, with the third application resulting in the greatest flux. In the periods 'B', no differences were found (P>0.05) between treatments. Indeed during these periods ('B'), the SL plots resulted in negative cumulative CH_4 fluxes, similar to those from the zero N and AN plots. Comparing the mean CH_4 cumulative fluxes from periods 'B' after fertilization, we found that CH_4 uptake by the soil was significantly lower after the third application compared with the first and second fertilization (P<0.001). The application of cattle slurry resulted in CH_4 emission factors

representing 0.01, 0.04 and 0.06% of the C applied following each application, and a total for the whole experiment of 0.04% C applied (kg CH_4-C/kg slurry-C).

Total cumulative CO_2 fluxes (Table 4.2) from the amendments were not statistically different to those from the zero N plots (P>0.05). The AN treatment resulted in significantly different mean CO_2 fluxes compared with the other treatments (Figure 4.5d) in the period 'B' after the first application (6^{th} -22^{nd} June) (P<0.05), with mean cumulative values of 80.8 kg CO_2-C ha^{-1} day^{-1}, 20% and 22% higher than zero N and SL, respectively.

4.3.6. Correlations between GHG fluxes and soil and weather parameters

The daily N_2O fluxes observed in all treatments showed significant positive correlation with daily %WFPS (r: 0.17; P<0.01), soil (r: 0.06; P<0.05) and air temperature (r: 0.08; P<0.05). Also, negative correlations were observed between the soil mineral N values and the corresponding N_2O fluxes observed from the treatments at each soil sampling day (NH_4^+-N: r:-0.17; P<0.01 and NO_3^--N: r:-0.13, P<0.05).

Daily CH_4 fluxes from zero N (r: 0.39; P<0.01) and AN plots (r: 0.21; P<0.01) were related to daily WFPS. Soil temperature was negatively correlated with the daily CH_4 fluxes from zero N and AN plots after the first (r: -0.25; P<0.01) and second fertilization (r: -0.22; P<0.01). Also, soil N-NH_4^+ contents were related to the corresponding CH_4 fluxes at the day of the soil sampling from zero N (0.40; P<0.01) and AN (0.23; P<0.01).

Daily CO_2 fluxes from all treatments showed significant correlations with daily soil temperatures after each fertilization (r values in each fertilization of 0.43, 0.34, 0.57; P<0.01) and also with soil WFPS (r values in each fertilization of 0.43, 0.34, 0.57; P<0.01).

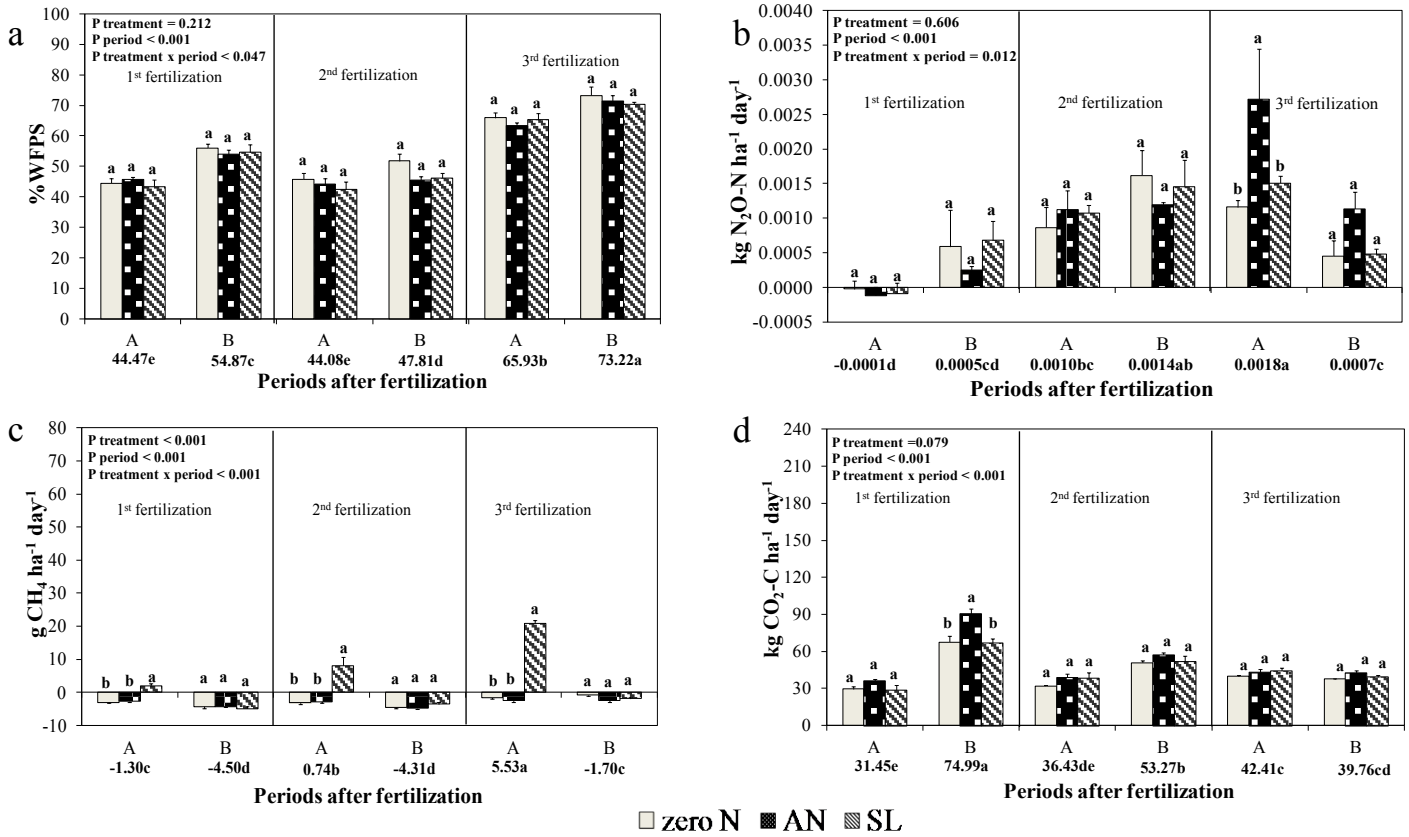

Figure 4.5. Results of a two way ANOVA repeated measures and pairwise multiple comparison test (Tukey test) for a) mean WFPS and mean cumulative fluxes of (b) N_2O, (b) CH_4 and (c) CO_2. Bars and error bars show the mean and standard error (n=3). Values below the x-axis show mean value of the periods ('A', 'B'). Means and bars with the same letter show no significant difference using Tukey test (P>0.05). Treatments: (zero N) control; (AN) ammonium nitrate; (SL) slurry. Periods - 1st Fertilization: 'A' (16th May-8th June) and 'B' (8th-22nd June); 2nd Fertilization: 'A' (22nd June- 16th July) and 'B' (16th July-6th September); 3rd Fertilization: 'A' (6th-30th September) and 'B' (30th September-13th November).

4.3.7. Losses of NH_3

On the basis of the output from the ammonia emission model (ALFAM), the slurry application under the warm and dry weather conditions of the experiment may have led to significant volatilization of NH_3. As some studies report (e.g. Pfluke et al. (2011); Thompson et al. (1990)) volatilization tends to occur within the first 24 hours after application to soil. Wind speed values used as the model input data were 5 m s^{-1} for the first application and 4 m s^{-1} in the other two fertilization events. With respect to air temperature, the surface broadcasting of slurry was carried out when mean air temperatures were 11, 14, 17 ºC, at the time of the first, second and third slurry applications. Values for DM and the NH_4^+-N content of the slurry applied in each fertilization (Table 4.1) were input into the ALFAM model. According to the model, the interaction of these input parameters may have caused losses of NH_3 of 86, 69 and 82% (% NH_4^+-N applied) after each slurry application, thus leaving little NH_4^+-N in the soil at risk of loss as N_2O or for plant offtake.

In the AN plots, the application of 80 kg N ha^{-1} of NH_4NO_3 (40 kg ha^{-1} as NH_4^+-N) may have resulted in a loss of 0.6 kg N ha^{-1} via NH_3 volatilisation after each fertilization.

4.3.8. Dry matter yield and N plant offtake

Dry matter yields were statistically greater (P<0.001) for the first two herbage cuts (22nd June and 24th August) than the third cut, for all treatments (Figure 4.6a). The application of AN and SL significantly increased DM in the first and second harvests (P<0.001) compared with the zero N plots. Dry matter yields in SL were 56% greater than yields from the zero N plots in August (P<0.001). No differences between treatments (P<0.001) were found for the DM yields after the third harvest (31st October). For each cut, the herbage N contents were similar between the zero N, AN and SL treatments. Highest total N herbage concentrations (mean value of 3.59% DM) were found in grass from the October harvest (Figure 4.6b), compared with the June and August harvests (P<0.001). So, N offtake (Figure 4.6c) in the first two applications were statistically greater (P<0.05) compared with the third application, for all treatments. Similar to DM yield, the AN plots significantly increased N offtakes in the first and second cut (p<0.05) compared with the zero N and SL plots. The SL treatment resulted in significantly greater N offtake (58% more) compared with N offtake on the zero N plots after the second harvest (P<0.05). At the October grass harvest, no differences in N offtake were found between N fertilization treatments (P>0.05).

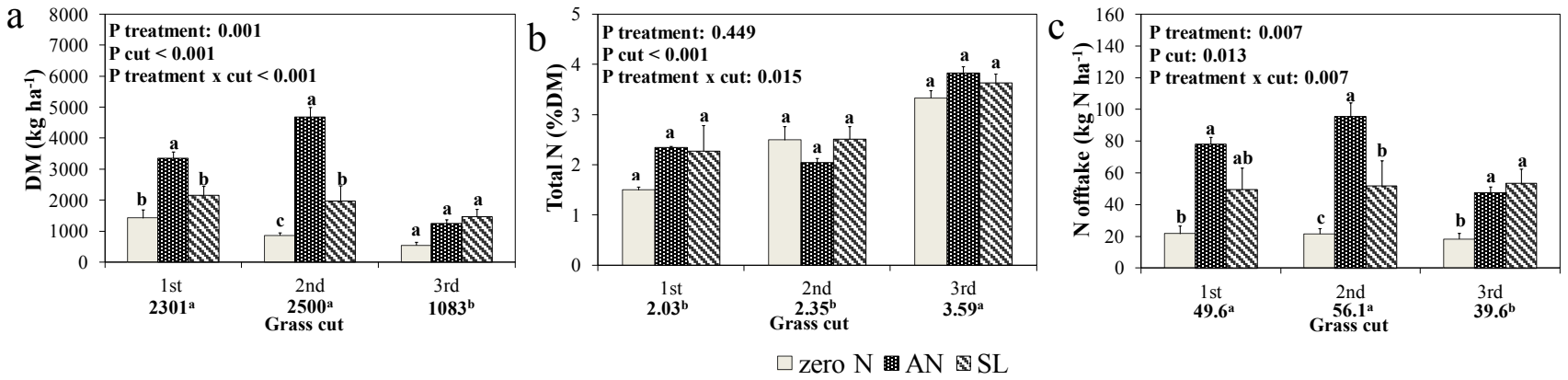

Figure 4.6. Results of a two way ANOVA repeated measures and pairwise multiple comparison test (Tukey test) for grass (a) dry matter (DM) yield expressed as kg ha^{-1}, (b) total plant N concentration expressed as %DM, and (c) N offtake (kg N ha^{-1}) in the three cuts carried out in the experiment. Values below the x-axis show the mean value of the treatments (zero N, AN, SL) within each grass cut (1^{st}, 2^{nd}, 3^{rd}). Means and bars with the same letter show no significant difference using Tukey test (P>0.05). Treatments: (zero N) control; (AN) ammonium nitrate; (SL) slurry. Grass harvests: 1^{st} (21^{st} June), 2^{nd} (24^{th} August), 3^{rd} (31^{st} October).

4.3.9. Nitrogen budget

Nitrogen inputs and outputs, measured or estimated (NH_3 volatilisation), for each period after fertilization and also for the whole experiment are summarized in the Table 4.3. The initial soil mineral N content for each period was taken into account to provide an indication of the available mineral N pool for plant uptake. The soil sample taken on 13th May prior to the first fertilization was considered as the initial soil N content for the first fertilization and for the entire experiment. For the second and third fertilization events, the soil mineral N values were from the last soil sample taken prior to the next fertilization.

Zero N plots showed a negative N balance after each of the three fertilization periods. The AN treatment resulted in a N deficit of 14.3 kg N ha^{-1} in the period studied after the second fertilization. In the first and third periods, surpluses of N were observed, with the highest surplus (33.5 kg N ha^{-1}) occurred after the third application. For the SL treatment, surpluses ranged from 3.7 to 9.8 kg N ha^{-1}. Across the entire measurement period (taking all three periods into account), only the zero N plots resulted in N deficit (negative values), unlike the AN and SL plots where soil remained with N surpluses of 21.6 and 15.5 kg N ha^{-1}, respectively.

Table 4.3. Initial soil N contents, N inputs and outputs (measured or estimated) and the N balance in the three periods studied after each fertilization, and for the whole experiment. Units expressed as kg N ha^{-1}. Treatments: (zero N) control; (AN) ammonium nitrate; (SL) slurry. 1st Fertilization: (16th May-22nd June); 2nd Fertilization: (22nd June-6th September); 3rd Fertilization: (6th-13th November); total experiment (16th May-13th November).

	1st fertilization			2nd fertilization			3rd fertilization			Total experiment		
	zero N	AN	SL	zero N	AN	SL	zero N	AN	SL	zero N	AN	SL
NH_4^+-N	1.8	2.0	1.5	1.2	1.5	1.3	1.2	1.4	1.3	1.8	2.0	1.5
NO_3^--N	1.5	1.9	1.5	0.0	0.0	0.0	0.0	0.1	0.2	1.5	1.9	1.5
Total initial soil mineral N content	3.3	3.9	3.0	1.2	1.5	1.3	1.2	1.5	1.5	3.3	3.9	3.0
Inputs												
NH_4^+-N	0.0	40.0	35.1	0.0	40.0	28.4	0.0	40.0	28.9	0.0	120.0	92.4
NO_3^--N	0.0	40.0	0.4	0.0	40.0	0.0	0.0	40.0	0.0	0.0	120.0	0.4
Organic-N	0.0	0.0	44.5	0.0	0.0	51.5	0.0	0.0	51.1	0.0	0.0	147.1
Total N inputs	0.0	80.0	80.1	0.0	80.0	79.8	0.0	80.0	80.1	0.0	240.0	240.0
Outputs												
N_2O-N	0.007	0.006	0.007	0.105	0.089	0.102	0.048	0.115	0.058	0.160	0.205	0.166
NH_3-N volatilization[a]	0.0	0.6	30.1	0.0	0.6	19.7	0.0	0.6	23.6	0.0	1.8	73.4
N plant offtake	21.7	77.9	49.3	21.6	95.2	51.5	18.3	47.3	53.1	83.3	220.4	153.9
Total N outputs	21.7	78.5	79.4	21.7	95.8	71.3	18.3	48.0	76.8	61.7	222.3	227.5
N balance	-18.4	5.4	3.7	-20.5	-14.3	9.8	-17.1	33.5	4.8	-58.4	21.6	15.5

[a] Estimated using the ALFAM model (Søgaard et al., 2002).

4.4. Discussion

4.4.1. Fluxes of N_2O

The low N_2O emissions observed in our experiment in 2011 were most likely the consequence of low %WFPS, high soil and air temperatures, and low available soil N contents. As already mentioned, 2011 was considered a very dry year compared with the 40-year mean for rainfall (Figure 4.1a), especially before and immediately after the first fertilizer application. The low rainfall after the first fertilization (20% less than the 40-year mean for May and June in the SW England) meant that the WFPS did not increase above 60%, resulting in frequent negative N_2O fluxes (as low as -0.005 kg N_2O-N ha^{-1} day^{-1}). Rain fell just before the second and third fertilizer applications, resulting in an immediate increase of the WFPS above 60% (the threshold value above which anaerobic activity becomes significant, Linn and Doran (1984)), and subsequent increases in N_2O fluxes (Davidson, 1993; Dobbie et al., 1999). Between the second and third applications, the WFPS varied between 35-60% producing low N_2O fluxes, probably as result of nitrification (Bateman and Baggs, 2005). The low N_2O emissions during period 'B' after the third fertilization were due to high %WFPS values (near saturation), which would have promoted complete denitrification to N_2 (Rudaz et al., 1999). In period 'A' after the third fertilization, where soil %WFPS was optimal for N_2O production (mean of 66% WFPS), there was only a small increase of up to 0.008 kg N_2O-N ha^{-1} day^{-1} from the AN treatment. But there was no increase in N_2O flux from the SL treatment, probably because of the lower NO_3^--N content in the SL treatment, and/or possibly because the addition of available C in the SL treatment enhanced full denitrification to N_2 (Weier et al., 1993).

The low N_2O fluxes observed during the 2011 experiment contrasted with previous experiments carried out in the South West of England. The North Wyke meteorological records show that during the past fifteen years (1995-2010), 2003 was a similar dry year, especially in the spring and summer. Smith et al. (2012) reported low N_2O emissions (0.15-0.22 kg N ha^{-1}) from cut grassland from an experiment on the same soil type in the same region in 2003, following applications of 80-100 kg N ha^{-1} as AN in spring and summer. They attributed these low N_2O emissions to the low % WFPS. However, their emissions in 2003 were still 25 and 2.5-fold greater than those measured in our experiment in 2011 after the applications in May and June, respectively. Smith et al. (2012) also reported an increase in N_2O emissions during the same months following NH_4NO_3 applications in 2004, which was considered a wet year, more typical of SW England. The amount of N_2O emitted in 2004 respect to 2003 was approximately 9 and 4- fold higher in the applications of May and June, respectively. Cardenas et al. (2010), also, reported frequent and high N_2O fluxes in 2006 (up to 200 g N_2O-N ha^{-1} day^{-1} in June) after the application of 75 kg N ha^{-1} as NH_4NO_3 in a grazed grassland on the

same soil type, close to our experimental site. They ascribed these high N_2O fluxes to intense denitrification due to the anaerobic soil conditions generated by the large amount of rainfall recorded. Chadwick et al. (2000) observed large differences in N_2O emissions following slurry application under different weather conditions at a site close to ours. They also observed that the amount of N_2O emitted was 10 times lower in July, under warmer conditions (WFPS values below 60%), than in April, probably due to the relatively dry (aerobic) nature of the soil. Unlike our experiment, the N_2O fluxes from the experiments cited above (on the same soil and conducted at the same time as our experiment) were not limited by soil mineral N, only by the WFPS. In this sense, the loss of NH_4^+-N through plant offtake and NH_3 volatilisation contributed to the low soil NH_4^+-N observed during our experiment and, therefore, low level of soil NO_3^-- N. In our study, N_2O emissions under the limited conditions of anaerobicity and soil NO_3^--N content resulted in emission factors lower than the IPCC default value of 1% (IPCC, 2006), and 8- and 40-fold lower than the lowest emission factors reported in the experiments carried out in North Wyke and cited above for NH_4NO_3 and cattle slurry, respectively.

4.4.2. Other N losses

Converting the emissions to proportion of total N, the ALFAM model estimated that 25-38% of the total N in the slurry was lost by NH_3 volatilization. These results contrasted with the estimated 1% following NH_4NO_3 application (Misselbrook et al., 2004b). The climatic conditions (warm and windy), and the application method (surface broadcasting) coupled with the high DM content of the slurry would have enhanced NH_3 volatilization from SL plots. Surface broadcasting of slurry results in greater NH_3 losses compared to other application methods such as band spreading, use of trailing hose or shallow injection (e.g. Huijsmans et al. (2003); Misselbrook et al. (1996); Pfluke et al. (2011); Smith et al. (2000)) due to the increased surface area of the slurry in contact with the air. Also, the absence of rainfall immediately after the slurry spreading reduced the infiltration of the slurry ammonium into the soil (Misselbrook et al., 2005). For the same reason, the high slurry DM content could have an important effect on NH_3 loss (Misselbrook et al., 2005; Pain et al., 1989); the DM content of the slurry used in our experiment was at the value suggested by Sommer and Olesen (1991), which promotes NH_3 emissions. The application under windy conditions would have enhanced the mass transfer and gas exchange between the slurry and the atmosphere (Misselbrook et al., 2005).

The warm and dry climatic conditions during the experiment stimulated plant growth on all treatments and thus generated a high plant N demand. For the zero N plots, the soil N deficits observed meant

that plant N requirement was probably met via the mineralisation of soil OM. For the SL fertilized plots, plant offtake represented 64% (mean value) of the total N applied in each fertilization. Moreover, plant N offtake seems to have been influenced by losses of N via NH_3 volatilization on the SL treatment, where the lowest plant offtake observed (after the first application) coincided with the highest NH_3 volatilization predicted, resulting in a much reduced soil NH_4^+-N content available for plant requirements. Misselbrook et al. (1996) reported data about NH_3 volatilization and N offtake from an experiment carried out at a sward located in SW England after surface application of cattle slurry. Their data showed that the lowest N offtake (23.4 kg N ha^{-1}) was in June coinciding with the highest NH_3 losses (30.6 kg N ha^{-1}). Also, Carran et al. (1982) observed that the soil conditions directly influence the proportion of N lost as NH_3 and N offtake by the plant. In the AN treatment, most of the N applied was taken up by the grass, especially after the first two harvests, when the plant N requirements are highest. In addition, after the second application the grass required more N than the N applied, suggesting mineralization of the OM supplied some the N required by the plant. The decrease in N offtake observed in the final harvest can be explained by the increase in soil %WFPS after the third fertilization. Losses of N could have limited the amount of N available for the plant. This effect was observed by Abassi et al. (2005) in a permanent grassland in Wales on soil in saturated conditions.

The N budget in the AN treatment showed a surplus (33.5 kg N ha^{-1}) at the end of the experiment. From this surplus, only 2.8 kg N ha^{-1} remained in the soil as mineral N. We assumed no losses of N via nitrate leaching during our measurement period, as there was little opportunity for leaching to occur. So, the surplus observed in AN could be attributed to losses as N_2 due to the anaerobic conditions caused by the high % WFPS after the third application. The resulting fraction of N applied attributed to N_2 (42% of the N applied) is higher than the range observed (8-25.5%) by Estavillo et al. (1994), Rudaz et al. (1999) and Velthof et al. (1996) after application of mineral fertilizers in grasslands. Compared to AN, the SL treatment did not result the same surplus due to the high plant N offtake and the estimated losses of N by NH_3 volatilization.

4.4.3. Fluxes of CH_4

Methane emissions were observed immediately after the application of slurry. Fluxes were short-lived, occurring within the first two (fertilization of June and September) to three days (in May) after application of the material. The larger mean CH_4 cumulative fluxes in the later applications could be related to the different DM content of the slurries applied, and the environmental conditions. The DM content of the slurry applied in September (6.5%) was lower than the DM of the slurry applied in

May. Lower DM contents could enhance slurry infiltration in the soil promoting methanogenesis due to greater anaerobicity. This was not the case for the June application compared to September because DM contents were similar. We suggest that soil moisture was the controlling factor in the CH_4 production due to lower % WFPS observed in the June application.

In the periods between application (periods "B") the soil acted as a net sink, resulting in negative fluxes as low as -9.8 g CH_4 ha^{-1} day^{-1}. Similar conclusions concerning the CH_4 fluxes from the soil after cattle slurry applications were reported by Chadwick et al. (2000), Jones et al. (2005) and Rodhe et al. (2006). Emissions of CH_4 from the AN plots were not observed, with negative fluxes often measured, indicating consumption of atmospheric CH_4. Soil moisture had an influence in gas diffusivity in the soil and, therefore, controlled the amount of CH_4 available for the oxidation by metanotrophic bacteria (Dunfield, 2007). In the periods following the first and second application, methanogenesis would have been suppressed in favour of methanotrophy due to the aerobic conditions generated by the low % WFPS in the zero N and AN plots. Pearson correlations between WFPS and CH_4 consumption rates observed in these treatments seem to confirm this assumption. Methane uptake rates generally increase with decreasing soil moisture because molecular diffusion in water is a factor of 10^4 times slower than in air (Borken et al., 2006). Higher soil temperature in May and June compared to September may have increased CH_4 oxidation rates. Methane consumption increases when soil is dry and warm (Price et al., 2003). The Pearson correlations between soil temperature and CH_4 oxidation rates confirmed this effect. Negative values showed CH_4 consumption was enhanced (became more negative) with the increase in soil temperature that occurred during the late spring and summer months. Some studies have shown that mineral N fertilizer application may decrease CH_4 consumption by an immediate inhibition of methanotrophs or change in the microbial community due to repeated applications (Bodelier and Laanbroek, 2004). In this sense, despite the positive correlations found between $N\text{-}NH_4^+$ and CH_4 comsumption values in the zero N and AN plots, there was no evidence of inhibition in this study.

4.4.4. Fluxes of CO_2

The CO_2 fluxes resulting from the grassland and measured by using static and opaque chambers represent ecosystem respiration. The application of slurries to the soil provides available C for soil microorganism activity, increasing of the microbial activity and resulting in large CO_2 fluxes after slurry application (Fangueiro et al., 2007; Meijide et al., 2010; Rochette et al., 2000a). However, in this experiment total CO_2 fluxes from soils fertilized with SL resulted in similar CO_2 fluxes from soils with zero N or amended with AN. These results suggest that soil CO_2 fluxes may have been induced

by the native soil C contents (Kuzyakov and Bol, 2006), rather than by the C contained in the slurry, and/or that another factor could have limited microbial respiration, not C.

The unusual warm and dry conditions observed during the experiment affected the pattern of soil CO_2 fluxes as the Pearson correlations confirmed, when relating soil temperature and moisture to soil CO_2 fluxes. The highest soil CO_2 fluxes following the first and second N applications coincided with high soil temperatures and dry soil conditions and contrasted with the low CO_2 rates observed after the third application, in autumn. The relationship between respiration and temperature has been reported in other studies. Meijide et al. (2010) observed in a barley field that the lowest CO_2 emissions occurred in late autumn and winter, when soil temperatures were below 10˚C. Hynšt et al. (2007), in a grassland affected by cattle impact in Czech Republic, and Jones et al. (2006), in a temperate grassland in Scotland, observed the opposite. They observed the highest CO_2 fluxes with warm temperatures. Soil moisture contents was also related to the CO_2 fluxes observed. Dry soil conditions observed after the first two applications resulted in greater CO_2 fluxes than those observed after the third fertilization, when high frequency of rainfall events was registered. In this sense, high WFPS may have suppressed soil respiration after the third fertilization by reducing soil CO_2 diffusivity rates (Rochette et al., 1991) and also by enhancing anaerobiosis and, in consequence, reducing aerobic respiration (Ball et al., 1999).

4.5. Conclusions

The unusual climatic conditions observed in 2011 in the SW England affected the pattern of fluxes of N_2O, CO_2, CH_4 during the experiment. Nitrification was probably the main pathway for N_2O production under the dry soil conditions (WFPS below 60%) observed during the first two applications. Large rainfall inputs identified after the third fertilization increased gradually soil %WFPS to saturation and could have enhanced conditions for complete denitrification. This situation also resulted in low N_2O fluxes from the SL and AN treatments. However, AN showed higher cumulative N_2O emissions compared with SL after the third application, probably due to greater anaerobicity and the lower N availability in the SL plots. High losses of N via NH_3 volatilization could have contributed to the low N_2O losses from the SL treatment, although these were modelled and not measured directly. Plant offtake of the applied N with both types of fertilizer also reduced the soil N pool available for potential N_2O production, especially during the first two applications. Methane emissions were recorded only from the slurry applications, and the increase of soil moisture enhanced methanogenic processes which occurred within 48 hours after the application. Carbon dioxide fluxes were influenced by soil temperatures and soil moisture contents, with the highest fluxes in the driest period of this study (May-June). The atypical weather during this experiment in some

sense offers an indication of the effect of future climate change, with decreases in the annual pattern of rainfall and increased temperatures in SW England, especially in late winter and early spring; all of which could result in a decrease of N_2O emissions.

CHAPTER 5. NITROUS OXIDE EMISSIONS FROM FORAGE MAIZE PRODUCTION ON A HUMIC CAMBISOL FERTILIZED WITH MINERAL FERTILIZER OR SLURRIES IN GALICIA, SPAIN

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Abstract

We conducted a field experiment in Galicia (NW Spain) to quantify N_2O emissions derived from fertilization practices carried out by local farmers growing forage maize (*Zea mays* L.). Forage maize was cultivated in a silt loam soil during 2008 and 2009, in different locations each year. Nitrous oxide fluxes were monitored during the whole growing season after the application of the following treatments: no N application (CN); 125 kg N ha^{-1} NPK at sowing and 75 kg granulated urea at top dressing (MN); 200 kg N ha^{-1} injected cattle slurry (CS) and 200 kg N ha^{-1} injected pig slurry (PS), with both slurry treatments applied at sowing. We observed that although fertilization significantly increased losses of N as N_2O, the type of fertilizer did not significantly affect the total cumulative N_2O emissions in either year. This could have been due to the high native soil C content in both experimental sites. Total cumulative N_2O emissions from N fertilized treatments ranged from 19.8 to 20.5 kg N ha^{-1} in 2008 and 10.8 to 11.7 kg N ha^{-1} in 2009, with the period between sowing and top dressing being the largest contributor. Nitrification probably caused the N_2O fluxes observed in the days following the application of N fertilizers in May, however the largest losses of N_2O were observed at optimal soil conditions for denitrification. Variations in N_2O fluxes between crop seasons could be attributed, in the first instance, to more N_2 rather than N_2O production during 2009, a consequence of the larger soil water filled pore space (>80%WFPS) during most of the sampling period. Another cause was probably the periods of soil rewetting observed in 2008, which triggered the largest N_2O fluxes observed and contributed to approximately 40% of the total cumulative N_2O emissions. The use of slurries or mineral fertilizer resulted in similar yield scaled N_2O emissions in both years. At a target N rate of 200 kg N ha^{-1} these were in the range 1.18-1.23 kg N_2O-N Mg^{-1} DM in 2008 and 0.51-0.58 kg N_2O-N Mg^{-1} DM in 2009. This study highlights the need to increase the knowledge of initial soil N contents at the moment of the N application and the dynamics of soil organic matter mineralization to adapt N rates to efficiently meet crop demands, especially in the period between sowing and top dressing application when demands are small.

5.1. Introduction

Nitrous oxide (N_2O) is a potent greenhouse gas that also plays an important role in the depletion of stratospheric ozone (O_3). The global warming potential of N_2O is 298 times that of the CO_2 in a 100-year time horizon (IPCC, 2007). Agriculture is the source of 10-12% of global anthropogenic greenhouse gases (GHGs), of which 60% is N_2O (Smith et al., 2007). Consistent with this trend, Spanish agriculture contributed approximately 76-78% to the total national N_2O produced in 2009 and 2010, 67% of which is from soil (MAGRAMA, 2012).

The application of organic and mineral fertilizers plays an important role in determining N_2O fluxes from soils (Mosier et al., 1998) via production from nitrification, the biological oxidation of ammonium (NH_4^+-N) to nitrate (NO_3^--N), and denitrification, the reduction of NO_3^--N to N_2. The contribution of these processes to N_2O production is controlled by soil oxygen (O_2) availability, mainly driven by soil water content. Generally any production at water filled pore space (WFPS) values below 60% is attributed to nitrification (Bateman and Baggs, 2005) whereas denitrification is the dominant process when WFPS exceeds 60% (Davidson, 1991). Large numbers of studies have considered denitrification to be the main N_2O producing process under high soil moisture, (Abbasi and Adams, 2000; Skiba and Ball, 2002) but N_2O fluxes can decrease when extremely anoxic conditions are generated in the soil (WFPS exceeds 80%) and N_2O is reduced to N_2 (Davidson, 1991). As Galicia is one of the wettest areas of Spain (>1000 mm $year^{-1}$), agricultural practices in this region may contribute greatly to anthropogenic N_2O emissions. Agriculture has great importance in Galicia, in the North West of Spain, especially forage maize production. It accounts for 76% (68,909 ha) of the total area of Spain dedicated to this crop (ESYRCE, 2013). This is because Galician agriculture is associated with the production and use of forage crops at the farm scale to maintain dairy cattle production systems. Maize irrigation is not required in Galicia due to the cool summers and high rainfall (39% annual rainfall is registered between May and October). However, the application of N based fertilizers is necessary to enhance crop yields. Slurries generated on Galician farms are commonly applied to avoid the cost of mineral fertilizers. Also, crops can take up N more efficiently from slurries due to the slow release of nutrients they contain. This characteristic of the slurry can also help to reduce environmental problems such as leaching and, if slurry is injected into the soil, the risk of losses of N from ammonia (NH_3) volatilization (Smith et al., 2000). However, the injection of the slurries could stimulate N_2O fluxes (Sistani et al., 2010; Wulf et al., 2002) as slurries contain easily decomposable organic carbon (C) which can trigger denitrification and also generate anaerobiosis in the soil by increasing biological O_2 demand (Rochette et al., 2000b).

The aims of this study were to understand the dynamics of the N_2O fluxes during the maize growing season under Galician climatic conditions and determine the influence of the type of fertilizer (injected slurries and mineral fertilizers) typically applied by local Galician farmers on the resulting emissions. We also aimed to identify what form of fertilization is associated with low N_2O production and high yields. This study will report N_2O emissions and crop yields from the maize growing seasons of 2008 and 2009, and present emission factors scaled by crop yields.

5.2. Material and methods

5.2.1. Location

Measurements were carried out for two consecutive growing seasons (2008 and 2009) on forage maize fields (*Zea mays* L.), which were established in different locations at Mabegondo Agricultural Research Centre (CIAM-INGACAL), Abegondo, Galicia, Spain (43.2425°N, 8.2608°W). The climate is European Atlantic (Fernández et al., 2011) and Mediterranean humid according to the agrologic classification provided by Papadakis (1966). The mean annual temperature for the period 1998-2007 was 13.1 ºC, and the mean minimum and maximum temperatures were recorded in February (2.9ºC) and August (25.4ºC), respectively. The mean rainfall for the same period was 1101 mm. The wettest month was October (177 mm) and the driest July (35 mm). The average rainfall registered during the forage maize growing season (average of the period May-September over the last 10 years) is 237 mm and the average temperature is 16.1ºC. The soil in both maize crop seasons was a Humic Cambisol with a silt loam texture in the top 10 cm. The soil properties measured in each location are shown in the Table 5.1.

Table 5.1. Properties of the upper 10 cm of the humic cambisol in the maize cropping sites studied.

	Sand	Silt	Clay[a]	Total C	Total N	C/N	pH	BD[b]
Year/Site	%			% DM[c]				Mg m^{-3}
2008	25	55	20	3.27	0.22	10.38	5.35	1.28
2009	19	56	25	3.60	0.35	10.22	5.30	1.25

[a] USDA classification

[b] BD: bulk density was calculated by using the empirical formula provided by Battilani et al. (1999)

[c] DM: dry matter

5.2.2. Experimental design

The experimental plots were established in May in both years as a randomized plot design with three replicates of four treatments: control without N fertilizer (CN), mineral fertilizer as NPK (15:15:15) and urea 46% (MN), cattle slurry (CS) and pig slurry (PS). Plots were 42 m^2 in area (4.2 m x 10 m) for control and mineral fertilizer plots. The area in those plots fertilized with cattle and pig slurries

was 88 m^2 (8.8 m x 10 m) to accommodate the 4.4 m application width of the slurry tanker used for the slurry injection.

5.2.3. Fertilization and slurry analysis

Mineral fertilizer was applied by hand in two doses at a final rate of 200 kg N ha^{-1}: 125 kg N ha^{-1} as NPK 15:15:15 fertilizer (7% nitrate N content, NO_3^--N; 7% ammonium N content, NH_4^+-N) before sowing (1st application, on 22nd May 2008 and on 22nd May 2009) and 75 kg N ha^{-1} as urea 46% for the top dressing when the plant was 40 cm high (2nd application, on 22nd July 2008 and 6th July 2009). Cattle slurry was from a storage pit located at CIAM and the pig slurry from a storage pit located on a private farm. Slurries were injected 20-25 cm into the soil at a target rate of 200 kg N ha^{-1} using a Joskin EMB tank with 8400 l of capacity and 40 cm band spacing injection. A sample of each slurry was analysed for total N prior to application to calculate an application rate equivalent to 200 kg N ha^{-1}. Cattle slurry was finally spread at rates of 66 and 69 m^3 $ha^{-1}$and pig slurry at rates of 62.5 and 76 m^3 ha^{-1} in 2008 and 2009, respectively (see slurries characteristics in Table 5.2). Before sowing, all plots were amended with P and K mineral fertilizers at a final rate of 100 kg P_2O_5 ha^{-1} and 250 kg K_2O ha^{-1}.

Slurry density was determined in fresh samples using a hydrometer (Nahita, Navarra, Spain). The pH of the slurry was measured with a pH meter (Crison GLP 22, Barcelona, Spain) after adding to 25 g of slurry to 25 ml of distilled water and stirring for 30 min. Slurry dry matter (DM) was determined after 24 h oven-drying at 105ºC. Organic matter (OM) was analysed in dry and ground (<0.01mm) samples using a thermogravimetric analyzer TGA-601 (LECO Corp., Michigan, USA). Analysis of N, P and K was determined in fresh samples using Kjeldahl digestion (AOAC, 2000). Total N was analysed by a colorimetric method (indophenol blue reaction) using a continuous flow analyzer AA III (Bran+Luebbe, Norderstedt, Germany). Total P was determined by the colorimetric reaction between orthophosphate and ammonium molydate to form phosphomolybdate blue and analysed by a UV-vis spectrophotometer (Thermo Scientific Evolution 201). Dry samples of slurry were analysed on a Leco TruSpec CN (Leco Corp., Michigan, USA) to determine total C contents. Slurry K, Ca, Mg, Na contents were determined by atomic absorption spectroscopy. Ammonium (NH_4^+-N) and nitrate (NO_3^--N) contents were extracted from fresh samples using 2M KCl. The extracts obtained were analysed using a segmented flow analyser AA III (Bran+Luebbe, Norderstedt, Germany).

Table 5.2. Chemical composition of the slurries applied in 2008 and 2009.

		2008		2009	
Property	Units	Cattle	Pig	Cattle	Pig
Density	g cm^{-3}	1.01	1.01	1.04	1.01
DM	%	7.30	2.13	6.57	2.22
pH	-	8.2	8.3	8.4	8.4
NH_4^+-N	%FM[a]	0.087	0.168	0.115	0.162
NO_3^--N	%FM	0.000	0.000	0.000	0.000
OM	g kg^{-1}DM	814	633	769	623
N	g kg^{-1}DM	42.0	119.5	43.3	110.3
P	g kg^{-1}DM	8.6	30.4	9.2	27.8
K	g kg^{-1}DM	44.1	84.9	47.3	83.2
Ca	g kg^{-1}DM	23.1	35.2	19.8	28.2
Mg	g kg^{-1}DM	6.1	18.2	6.9	14.7
Na	g kg^{-1}DM	6.1	13.2	3.9	11.3

FM; fresh weight

5.2.4. Sowing, harvesting and plant analysis

Maize (*Zea mays* L., variety DKC3745) was planted on 22nd May 2008 and 22nd May 2009, with a 75 cm and 14 cm row and plant spacing, respectively. Planting rate was approximately 90,000 seeds ha^{-1}. Plots were harvested on 16th October 2008 and 29th September 2009. At the optimal maturity stage (the milk line is half to two thirds down the kernel), two central lines per plot were selected. In each line, 10 plants in a stretch of 7 m were cut with a sickle to 10-15 cm from the root and weighed. Samples of 300 g were selected for analysis of the percentage dry matter (%DM), measured by oven-drying at 80ºC for 18 h until constant weight. Dry samples were ground to pass a sieve of 1 mm and scanned on a near infrared reflectance spectrophotometer, NIRS Systems 6500 (FOSS NIRS Systems, Inc., Silver Spring, Washington, USA) for analysis of crude protein (CP) at 2 nm intervals between 1100 and 2500 nm. Spectral data were processed using WINISI software (ISI, Port Matilda, PA).

5.2.5. Nitrogen use efficiency

Dry matter yield and crude protein contents were used to calculate N uptake in plant tissue by the following equation ((Eq.1)):

$$N\ uptake: DM \times CP/\ 100/\ 6.25 \quad \text{(Eq.1)}$$

where *DM* is the dry matter (kg ha^{-1}), *CP* is the crude protein content (in %DM) and 6.25 is the conversion factor of protein to N content (MacDonald et al., 2002).

Apparent nitrogen recoveries (*ANR*) and apparent nitrogen efficiency (*ANE*) were calculated for each N treatment by the following equations (Eqs. (2) and (3)):

$$ANR\ (\%): 100 \times (N\ uptake\ fert - N\ uptake\ unfert)/N\ fertilizer \quad \text{(Eq.2)}$$

$$ANE\ (\text{kg DM kg}^{-1}\ \text{N}): (DM\ fert - \text{DM}\ unfert)/N\ fertilizer \quad \text{(Eq.3)}$$

where *N uptake fert* and *N uptake unfert* denote the above-ground plant N uptakes in the fertilized and control plots (kg N ha^{-1}), respectively and *DM fert* and *DM unfert* are the yields in fertilized and control plots (Mg ha^{-1}), respectively.

5.2.6. Measurements of N_2O fluxes-chamber design and operation

Nitrous oxide fluxes were measured using the closed chamber technique (Ryden and Rolston, 1983). Chambers were cylinders of polyvinyl chloride (PVC) with a volume of 0.018 m^3 (height: 36 cm; diameter: 25 cm) with a lid fitted with a rubber septum as a sampling port. Two chambers per plot (i.e., six chambers per treatment) were placed between rows and left in the same position during the experiment. On 30th May 2008 and 5th June 2009, before the application of the herbicide (4 l ha^{-1} as Acetochlor 45%+Terbuthylazine 21.4% (Harness GTZ)) and the insecticide (1 l ha^{-1} as Clorpyrifos 46%), chambers were removed to facilitate spreading and to avoid chamber contamination. After these applications they were re-inserted into the soil in the same position and sampling restarted 24 h later to avoid the effect of disturbance on the soil-atmosphere-gas transfer (Parkin and Venterea, 2010).

5.2.7. Nitrous oxide fluxes

Soil N_2O fluxes were measured a total of 70 times between 22nd May and 16th October 2008 and 45 times between 22nd May and 25th September 2009, and always between 09:00 and 10:00 h, which is

the period of time considered to be representative of the daily mean (Alves et al., 2012). During the growing seasons of 2008 and 2009 and after the N applications, gas samples were taken three or five times a week. Chambers were closed for 60 min (T_{60}), the period of time where the headspace N_2O accumulation is linear (Chadwick et al., 2014). After this, a sample of the chamber headspace was withdrawn through the rubber septum using a 10 ml polypropylene syringe fitted with a hypodermic needle. An ambient sample was also taken to provide background values for N_2O (Cardenas et al., 2010; Chadwick et al., 2014) and the time zero sample (T_0). Each sample was transferred into a pre-evacuated 10 ml vial for storage and analysis. A sample of 12 ml was injected to over-pressurize the vials, preventing a back-diffusion of air (Hyde et al., 2006). The N_2O concentration of the samples was analysed in the laboratory using a gas chromatograph Thermo Finnigan Trace gas chromatograph (GC 2000) fitted with an electron capture detector (ECD) at 330ºC and HP-Plot Q column (30 mm × 0.3 mm). Nitrous oxide fluxes were calculated using the following equation (Eq. (4)):

$$F = \rho \times V/A \times (Ct - C_0)/t \times (273.15/T) \quad \text{(Eq. 4)}$$

where F is the N_2O flux (μg N_2O-N m^{-2} h^{-1}); ρ the gas density (1.26 x 10^9 μg m^{-3} N_2O-N) under STP conditions (273 K and 101,325 Pa); V and A are the volume (0.02 m^3) and area (0.0491 m^2) of the chamber; C_{60} and C_o (μm^3 m^{-3}) are the N_2O concentrations 60 min after chamber closure (T_{60}) and the ambient sample (T_0), respectively; t is the time of chamber closure in hours (T_{60}); and T is the air temperature (K) at the time of sampling. Nitrous oxide fluxes were transformed to daily fluxes (kg N_2O ha^{-1} day^{-1}) for the calculation of the cumulative N_2O emissions. Nitrous oxide fluxes for dates between samplings were interpolated and the cumulative emissions calculated using the trapezoidal method (Cardenas et al., 2010; Louro et al., 2013). In order to obtain the cumulative N_2O emissions and the standard error for each treatment, cumulative N_2O emissions per plot (2 chambers) were calculated and the values obtained from the tree plots averaged.

Based on the N-applications and because of the split application of mineral fertilizer, cumulative N_2O emissions were calculated in two periods within each cropping season. In 2008 periods were: '2008I' (22nd May-22nd July) and '2008II' (22nd July-16th October). In 2009: '2009I' (22nd May-6th July) and '2009II' (6th July-25th September) (see Figure 5.1).

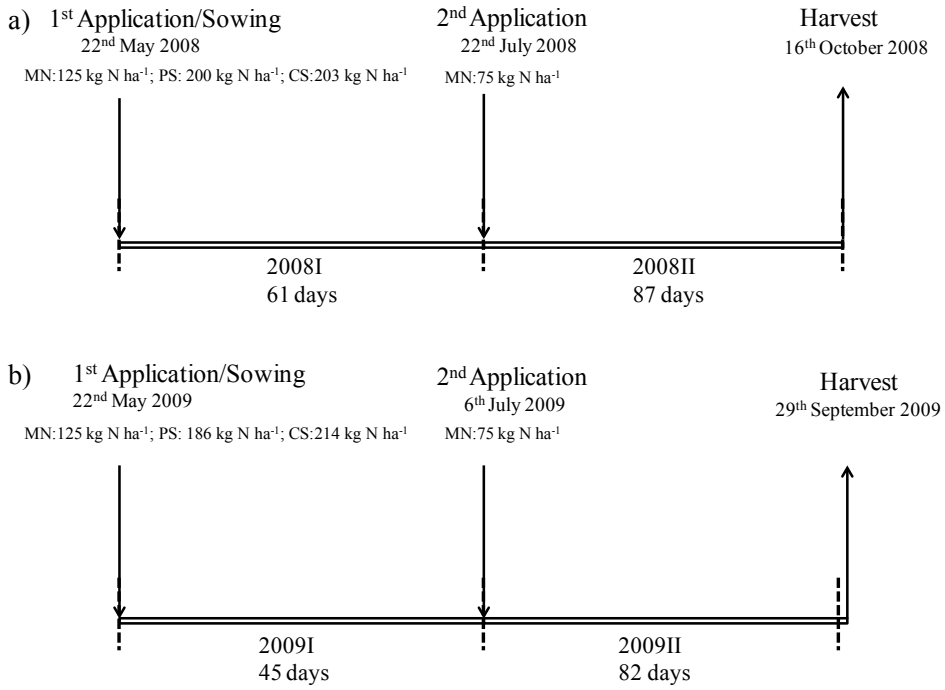

Figure 5.1. Timeline and periods considered in a) 2008 and b) 2009.

Total cumulative N_2O emissions from the control treatment were subtracted from the corresponding values in N fertilized treatments during the whole period of measurements, giving net total N_2O emissions as a response to the N applied for each N fertilizer in each cropping season. Emission factors (EFs) for each N fertilizer were calculated by dividing the net N_2O cumulative emissions by the total N fertilizer applied and expressing it as a percentage. The assessment of the impact of cropping practices on greenhouse gas (GHG) production per unit yield is important for maintaining high yields with minimal GHG emissions (Van Groenigen et al., 2010). Yield scaled N_2O emissions were calculated by dividing the total N_2O cumulative emissions in each treatment by their resulted crop yields and expressing them in kg N_2O-N Mg^{-1} DM.

5.2.8. Soil sampling and analysis

Soil samples at 10 cm depth were collected throughout both cropping seasons for the analysis of mineral N contents (ammonium (NH_4^+-N) and nitrate (NO_3^--N)) and soil moisture. Soil NH_4^+-N and NO_3^--N were determined by extracting 100 g of fresh soil with 200 ml 1M KCl. Extracts were analysed colorimetrically for NH_4^+-N and NO_3^--N soil contents using a segmented flow auto-analyser AA III (Bran+Luebbe, Norderstedt, Germany). Soil moisture content was determined gravimetrically after oven- drying at 105ºC for 24 h. Porosity was calculated from the bulk density (BD) at each site

by assuming a particle density of 2.65 Mg m^{-3}. Water filled pore space (WFPS) was calculated by dividing the soil moisture content and BD by the porosity and expressing it as a percentage.

5.2.9. Meteorological data

Daily rainfall (mm) and daily mean air and soil (10 cm depth) temperatures (ºC) were obtained from the weather station located at CIAM which includes a pluviometer (Thies Clima, Germany, model 5.4032.35.007) and air (Geonica, Spain, model STH-5031) and soil (Campbel Scientific Spain, model T-107) temperature probes. Monthly mean rainfall and air temperatures for the last 10 years (1998-2007) and the corresponding means for 2008 and 2009 were also used.

5.2.10. Statistical methods

The statistical analyses were performed using Sigmaplot 11. Total cumulative N_2O emissions were transformed into mean cumulative emissions by dividing the total cumulative emissions by the number of days. This allowed comparisons to be made between periods and years that differed in numbers of days. Two way repeated measures analysis of variance (ANOVA) was used to determine significant differences between the periods within each year (i.e. periods ˈ2008Iˈ versus ˈ2008IIˈ and ˈ2009Iˈ versus ˈ2009Iˈ) and between the years themselves (i.e. 2008 versus 2009). In each of these three analyses the treatment factor (CN, MN, CS and PS) was also included to determine a significant effect on the mean cumulative N_2O emissions. The same test was also performed to determine differences in EF within each year and between years. For total cumulative N_2O emissions, one way ANOVA was used to test for differences between treatments (CN, MN, CS and PS) within each period and also within each year. For WFPS and mineral N contents, two way repeated measures ANOVA was used to test for significant differences between treatments (CN, MN, CS and PS) and between soil sampling days within each period and year. For plant yield, N uptake, ANR, ANE and yield-scale N_2O emissions, one way ANOVA was used to investigate differences caused by the treatments (CN, MN, CS and PS). Tukey's test was used to determine which treatment groups were different at the $P \leq 0.05$ significance level.

5.3. Results

5.3.1. Weather conditions

Daily mean air temperature from the day of seeding to harvest was similar in both years, ranging from 12 to 25ºC in 2008 (Figure 5.2a) and from 11 to 24 ºC in 2009 (Figure 5.2b). Daily mean soil temperatures were also similar with ranges of 13-22 ºC in 2008 and 13-21 ºC in 2009. Total rainfall during the sampling periods in 2008 and 2009 (Figure 5.2a) was 360 mm (167 mm in ˈ2008Iˈ and 193 mm in ˈ2008IIˈ) and 296 mm (145 in ˈ2009Iˈ and 151 mm in ˈ2009IIˈ), respectively. The first fertilization in both years was followed by high rainfall for the first 12 days in '2008I' and for the first 19 days in '2009I'. In '2008I', 11 days before the second fertilization, 28 mm of rain fell after a long period with no significant rainfall. The second N fertilization in both cropping seasons (periods ˈ2008IIˈ and ˈ2009IIˈ) was not followed by large rainfall. In '2008II', large rainfall was observed between days 25 and 46; in '2009II' most of the rain fell in one single event on day 15.

5.3.2. Water filled pore space (WFPS)

The WFPS values reported in this study are approximate as they were derived using an estimated BD which was based on the sand and clay contents of the soil at each site (Battilani et al., 1999). We have carried out the statistical analysis of the treatments using this parameter but also provide the equivalent measured gravimetric soil moisture content (expressed as % water) as it is a more accurate descriptor of moisture for our study (Figures 5.2c, 2d). No significant differences in soil WFPS between treatments were observed on any sampling day within either year (P>0.05). Mean soil WFPS during '2008I' was 73% with the largest WFPS values in the following 25 days after the first fertilization (Figure 5.2c). Also, in '2008I', the rain observed 11 days before the second fertilization increased soil moisture levels from approximately 50% to almost 60%WFPS. The beginning of the ˈ2008IIˈ period was characterized by dry conditions (as low as 30% WFPS) until the rainfall between days 25 and 46 increased WFPS to a mean value of 80%. In '2009I', the soil remained saturated (100% WFPS) or with standing water at the soil surface (WFPS>100%) (Figure 5.2d). In ˈ2009IIˈ, throughout the period mean soil WFPS was 77% except for a couple of sampling days after day 15 where levels reached values >100%WFPS.

5.3.3. Soil mineral N

5.3.3.1. Soil ammonium contents

In '2008I', soil NH_4^+-N levels (0-10 cm) in N-fertilized plots were not significantly higher as those in CN plots (Figure 5.3a) (P>0.05). In '2008II', NH_4^+-N values from MN plots were larger than the other treatments during the first 36 days (P<0.05). In '2009I', significant differences between the N-treatments and the CN treatment were observed in the first 26 days (P<0.05) (Figure 5.3b). The largest soil NH_4^+-N contents were observed immediately after the first fertilization. In ˈ2009IIˈ, soil NH_4^+-N contents in MN plots were significantly larger than the other treatments during the first 23 days (P<0.05).

5.3.3.2. Soil nitrate contents

In both years, soil NO_3^--N contents (0-10 cm) in the CN plots were in general lower than the other treatments (P<0.05). However, short-lived increases in NO_3^--N levels from CN were observed in both years even when no N fertilizer was applied. During 2008, CN plots reached levels up to 61 kg N ha^{-1} on day 33 in '2008I' and 74 kg N ha^{-1} on day 6 in '2008II' (Figure 5.3c). Maximum soil NO_3^--N contents of 97 and 118 kg N ha^{-1} were reached on day 21 in period '2008II' from CS and PS plots, respectively. In MN plots, fertilizations resulted in maximum soil NO_3^--N contents of 121.5 kg N ha^{-1} (on day 18 in ˈ2008Iˈ) and 93.1 kg N ha^{-1} (on day 13 in ˈ2008IIˈ). In 2009, the largest soil NO_3^--N contents observed in CS and PS plots (114 and 204 kg N ha^{-1}, respectively) were reached on days 31 and 38 in ˈ2009Iˈ, respectively. In the MN plots, although a similar pattern to the CS and PS plots was observed, the maximum soil NO_3^--N content (205.2 kg N ha^{-1}) was observed on day 11 in period ˈ2009IIˈ.

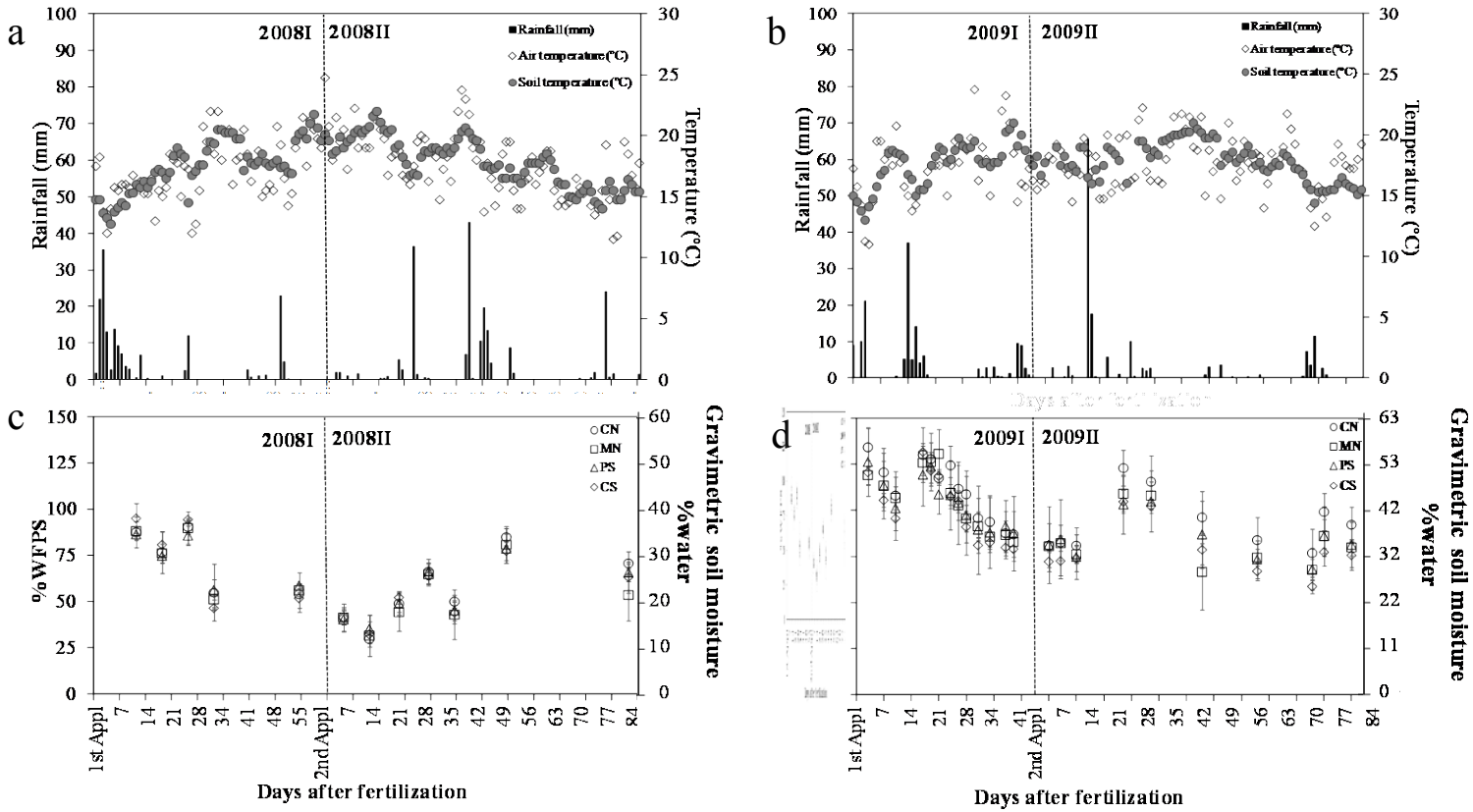

Figure 5.2. Daily rainfall, soil temperatures and air temperatures in a) 2008 and b) 2009. Filled bars represent daily rainfall and unfilled and filled circles represent daily air and soil temperatures, respectively. Soil water filled pore space (%WFPS) and gravimetric soil moisture contents (%water) following fertilizations in c) 2008 and d) 2009. Data points and error bars represent mean value (n=3) and standard error, respectively. Vertical lines mark periods between N applications. Treatments were: control (CN), mineral fertilizer as NPK+ granular urea 46% (MN), injected pig slurry (PS), injected cattle slurry (CS). Periods were: '2008I' (22nd May-22nd July), '2008II' (22nd July-16th October), '2009I' (22nd May-6th July) and '2009II' (6th July-25th September).

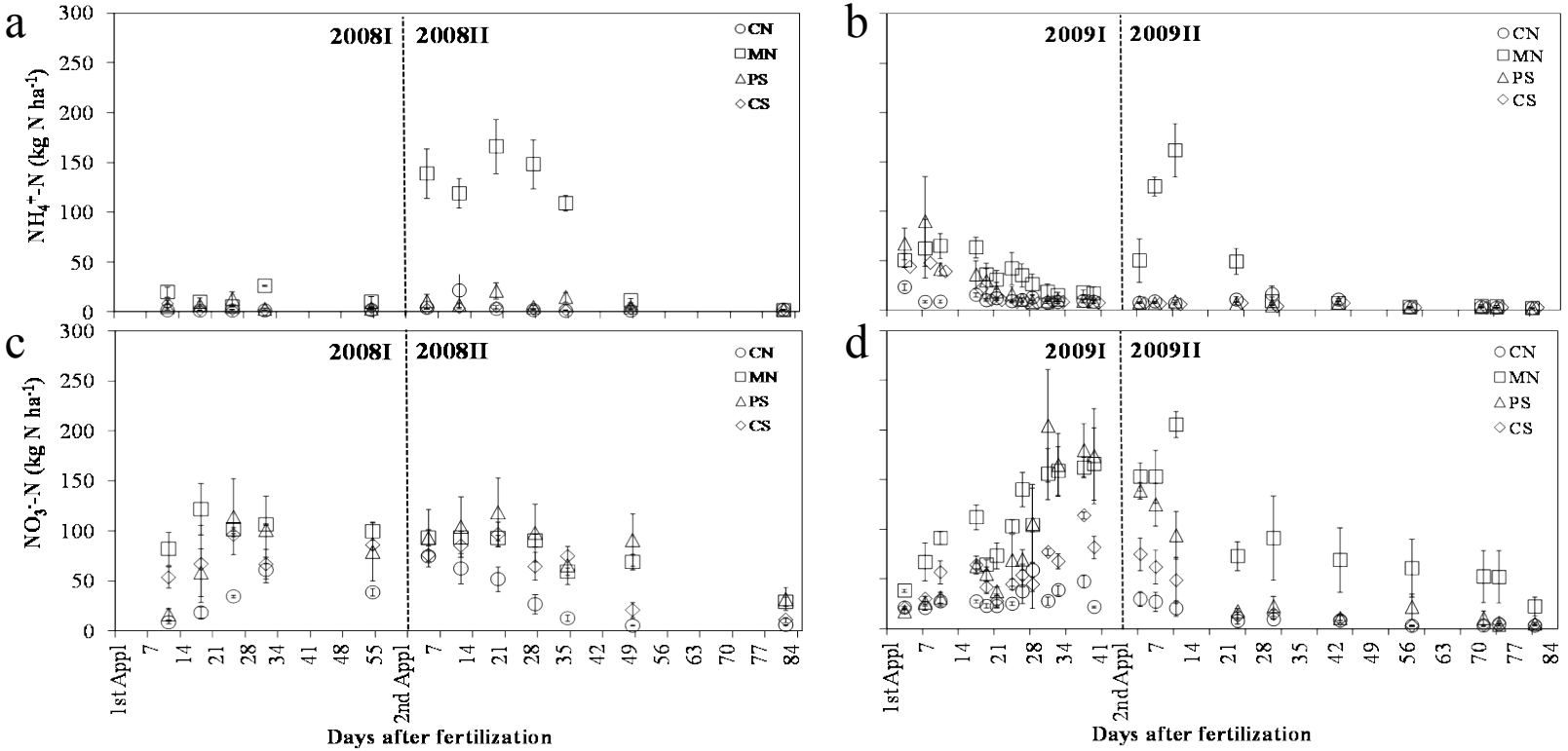

Figure 5.3. Soil mineral N contents following N applications in 2008 and 2009. Soil NH_4^+-N in 2008 (a) and 2009 (b). Soil NO_3^--N contents in 2008 (c) and 2009 (d). Data points and error bars represent mean value (n=3) and standard error. Vertical lines mark periods between applications. Treatments were: control (CN), mineral fertilizer as NPK+ granular urea 46% (MN), injected cattle slurry (CS) and injected pig slurry (PS). Periods were: '2008I' (22nd May-22nd July), '2008II' (22nd July-16th October), '2009I' (22nd May-6th July) and '2009II' (6th July-25th September)

5.3.4. Nitrous oxide fluxes

In 2008, N_2O fluxes were large and frequent, even from CN plots (Figure 5.4a). In ˈ2008Iˈ, the largest N_2O fluxes were observed between days 51 and 55 from N-fertilizer treatments (1933, 2035, 1647 μg N_2O-N m^{-2} h^{-1} in MN, CS and PS, respectively) and on day 41 from CN plots (1137 μg N_2O-N m^{-2} h^{-1}). The largest N_2O peaks of the experiment from all treatments (range of 1772-2313 μg N_2O-N m^{-2} h^{-1}) were observed between days 27 and 41 in period ˈ2008IIˈ. In 2009, N_2O fluxes were less frequent and lower than in 2008. The largest N_2O fluxes in all treatments (range of 939-1642 μg N_2O-N m^{-2} h^{-1}) were reached on day 19 in ˈ2009Iˈ. In ˈ2009IIˈ, the largest N_2O fluxes from the treatments (range of 751-992 μg N_2O-N m^{-2} h^{-1}) were between days 28 and 32.

5.3.5. Cumulative N_2O emissions

As Table 5.3 shows, N-based fertilizers significantly increased total cumulative N_2O emissions compared to the CN plots (P<0.001) in both years and in the periods studied within each year (P<0.001) but no differences between the forms of N applied were observed (P>0.05). The patterns of the cumulative N_2O emissions from the treatments were similar to those from CN until day 55 in ˈ2008Iˈ (Figure 5.4c) and day 21 in ˈ2009Iˈ(Figure 5.4d), when N applications progressively increased losses compared to CN. Comparing losses in the two periods studied within each year, treatments resulted in mean cumulative N_2O emissions 1.4-fold larger inˈ2008Iˈ than in ˈ2008IIˈ and 2-fold greater in ˈ2009Iˈ than in ˈ2009IIˈ (P<0.001). Comparing years, in 2009 all treatments showed significantly lower mean total cumulative N_2O emissions than the corresponding values in 2008 (P<0.001). In terms of EF, no significant differences between treatments were observed within each year and between years (P>0.05). Losses of N_2O in relation to the N applied to the forage maize were 1.81% (MN), 1.63% (PS) and 1.74% (CS) (mean of both years).

Table 5.3. Nitrogen applied (kg N ha^{-1}), mean (kg N_2O-N ha^{-1} day^{-1}) and total (kg N_2O-N ha^{-1}) cumulative N_2O emissions in the periods studied after each N application and for the maize total growth season and the resulting emission factors in 2008 and 2009. Treatments were: control (CN), mineral fertilizer as NPK+ granular urea 46% (MN), injected cattle slurry (CS) and injected pig slurry (PS). Mean values of three replicates and standard error between brackets. Treatments with different letters within each column and year are significantly different (P<0.05)

		'I'			'II'			Total ('I'+'II')			
Year/Site	Treatment	N applied	Mean N_2O cumulative fluxes	Total N_2O cumulative fluxes	N applied	Mean N_2O cumulative fluxes	Total N_2O cumulative fluxes	Total N applied	Mean N_2O cumulative fluxes	Total N_2O cumulative fluxes	EF
2008		22nd May-22nd July 2008			22nd July-16th October 2008			22nd May-16th October 2008			
	CS	203	0.17 (0.01)[a]	10.5 (0.6)[a]	-	0.12 (0.01)[a]	10.0 (0.7)[a]	203	0.14 (0.00)[a]	20.5 (0.8)[a]	2.15 (0.53)[a]
	PS	200	0.16 (0.01)[a]	9.8 (0.4)[a]	-	0.12 (0.01)[a]	10.0 (0.8)[a]	200	0.13 (0.00)[a]	19.8 (0.4)[a]	1.82 (0.28)[a]
	MN	125	0.16 (0.01)[a]	9.9 (0.6)[a]	75	0.11 (0.00)[a]	9.9 (0.2)[a]	200	0.13 (0.00)[a]	19.8 (0.8)[a]	1.80 (0.07)[a]
	CN	-	0.13 (0.00)[b]	8.1 (0.3)[b]	-	0.09 (0.00)[b]	8.1 (0.3)[b]	-	0.11 (0.00)[b]	16.2 (0.8)[b]	-
2009		22nd May-6th July 2009			6th July-25th September 2009			22nd May-25th September			
	CS	214	0.13 (0.01)[a]	5.8 (0.2)[a]	-	0.06 (0.01)[a]	5.2 (0.4)[a]	214	0.09 (0.00)[a]	10.9 (0.4)[a]	1.33 (0.35)[a]
	PS	186	0.12 (0.00)[a]	5.5 (0.2)[a]	-	0.06 (0.00)[a]	5.3 (0.1)[a]	186	0.08 (0.00)[a]	10.8 (0.2)[a]	1.44 (0.35)[a]
	MN	125	0.14 (0.01)[a]	6.5 (0.4)[a]	75	0.06 (0.00)[a]	5.2 (0.3)[a]	200	0.09 (0.01)[a]	11.7 (0.7)[a]	1.82 (0.45)[a]
	CN	-	0.09 (0.01)[b]	4.2 (0.5)[b]	-	0.04 (0.00)[b]	3.8 (0.2)[b]	-	0.06 (0.00)[b]	8.1 (0.5)[b]	-

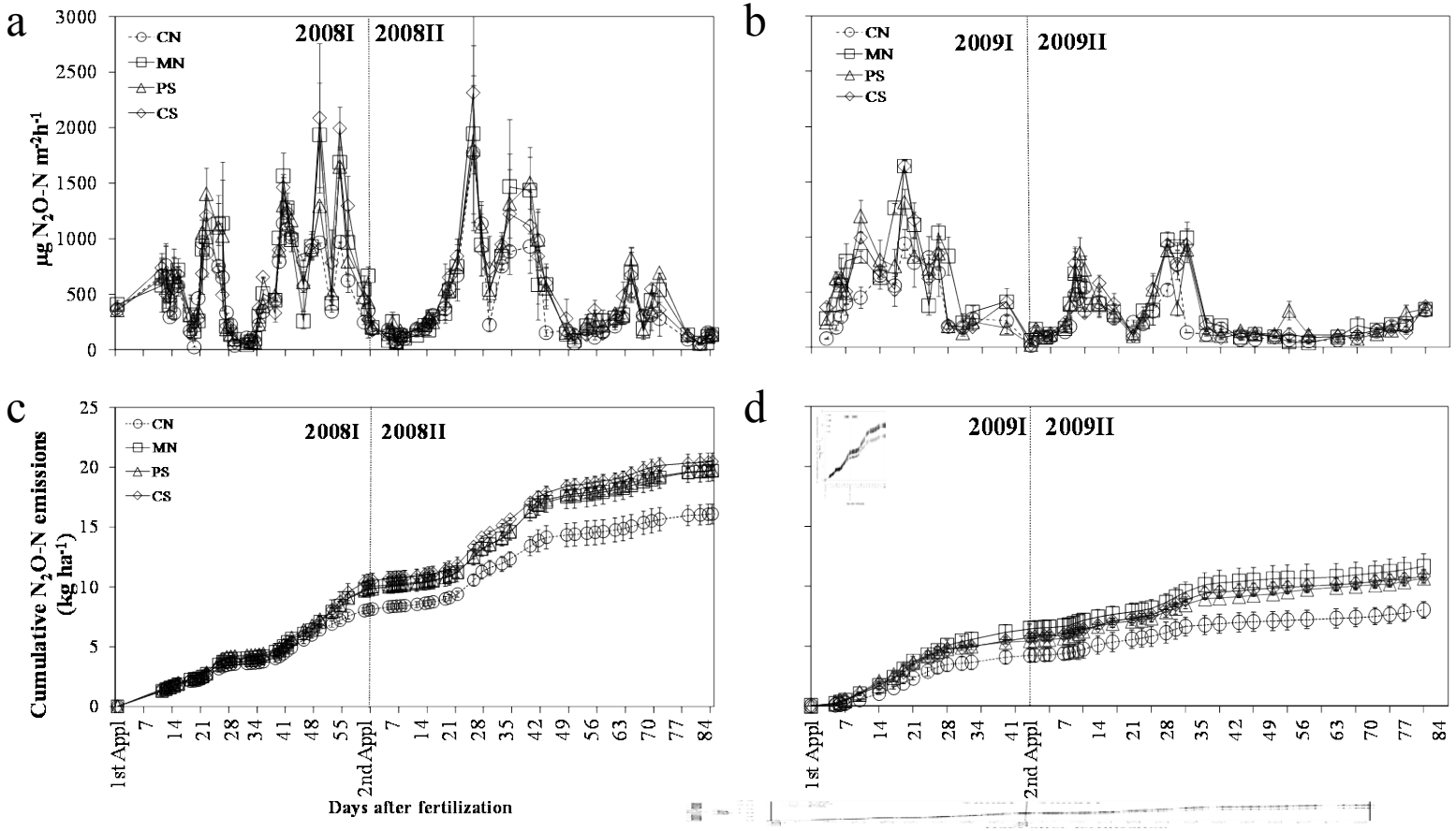

Figure 5.4. Nitrous oxide fluxes after N fertilization in a) 2008 and b) 2009. Cumulative N_2O emissions in c) 2008 and d) 2009. Data points and error bars represent the mean value of three replicates and the standard error of the mean, respectively. Vertical lines mark periods between applications. Treatments were: control (CN), mineral fertilizer as NPK+ granular urea 46% (MN), injected cattle slurry (CS) and injected pig slurry (PS). Periods were: '2008I' (22nd May-22nd July), '2008II' (22nd July-16th October), '2009I' (22nd May-6th July) and '2009II' (6th July-25th September)

5.3.6. Forage maize production and N uptake

In 2008, the application of N fertilizers resulted in similar DM yields and crop N uptake compared to maize plots with no N fertilizer application (P>0.05) (Table 5.4), with overall mean values of 16.4 Mg DM ha^{-1} and 163.5 kg N ha^{-1} for DM yield and N uptake, respectively. In 2009, DM yields and N uptake from N fertilized treatments were 1.5 and 2-fold larger than for the CN treatment (P<0.05) but no significant differences in these parameters between N-fertilizers were observed. In terms of ANE and ANR (Table 5.4), no significant differences between N-fertilized treatments were observed in any year (P>0.05). However, ANE and ANR values in 2009 were 2.6 fold and 2.0-fold larger than the corresponding averages in 2008, respectively. The yield-scaled N_2O emissions (Table 5.4) showed no differences between N fertilized and CN plots in any year (P>0.05) but values in 2009 were approximately 2-fold lower than those obtained in 2008 (P<0.05).

Table 5.4. Dry matter yield, N uptake, apparent N efficiency (ANE), apparent N recovery (ANR) and yield-scale N_2O emissions in the maize growth seasons of 2008 and 2009. Treatments were: control (CN), mineral fertilizer as NPK+ granular urea 46% (MN), injected cattle slurry (CS), injected pig slurry (PS). Mean values of three replicates and standard error between brackets. Treatments with different letters within each column and year are significantly different (P<0.05)

Year/Site	Treatment	N applied	Yield	N uptake	ANE	ANR	yield-scaled N_2O fluxes
		kg N ha^{-1}	Mg DM ha^{-1}	kg N ha^{-1}	kg DM kg^{-1} N	%	kg N_2O-N Mg^{-1} DM
2008	CS	203	18.3 (2.7)[a]	175.2 (17.7)[a]	19.65 (10.61)[a]	23.01 (4.70)[a]	1.18 (0.19)[a]
	PS	200	16.5 (1.2)[a]	177.0 (11.8)[a]	10.82 (3.98)[a]	22.12 (6.53)[a]	1.22 (0.09)[a]
	MN	200	16.4 (1.4)[a]	169.2 (9.8)[a]	10.27 (4.79)[a]	18.25 (3.16)[a]	1.23 (0.14)[a]
	CN	-	14.3 (0.5)[a]	132.7 (12.6)[a]	-	-	1.13 (0.07)[a]
2009	CS	214	20.6 (1.0)[a]	161.5 (10.8)[a]	32.58 (8.41)[a]	34.45 (8.86)[a]	0.53 (0.05)[a]
	PS	186	21.0 (0.4)[a]	176.1 (8.7)[a]	39.44 (5.79)[a]	47.48 (8.06)[a]	0.51 (0.01)[a]
	MN	200	20.2 (0.6)[a]	189.7 (10.3)[a]	32.93(3.73)[a]	50.93 (3.61)[a]	0.58 (0.05)[a]
	CN	-	13.7 (1.2)[b]	87.8 (13.2)[b]	-	-	0.59 (0.02)[a]

5.4. Discussion

5.4.1. Influence of soil mineral N and moisture contents on N_2O fluxes

We assessed different N-fertilizers typically applied by local farmers during forage maize cultivation in order to identify the N fertilizer type that related to low N_2O emissions while maximizing production. However, the experimental sites had large initial mineral N contents in the soil profile (106 kg N ha^{-1} in 2008 and 123 kg N ha^{-1}, in 2009, data not shown). Low C/N residues from previous crops (pea/pea-triticale) were incorporated in both sites one year prior the maize season, followed by a fallow period during winter. So, the application of the different fertilizers at sowing further increased the mineral-N pool in the soil. This explains the high N_2O background values and those induced by the application of fertilizers observed throughout both cropping seasons. The losses of N_2O were especially high during the first period of each year e.g.'2008I' and '2009I' as the N plant requirements were low at this growth stage and soil WFPS and soil mineral N contents were high (Drury et al., 2014; Sehy et al., 2003; Tan et al., 2009). However, the total losses varied between years, probably due to the different soil WFPS (Figures 5.2c, 2d). These differences could have also been related to the different positions on the slope of the experimental sites; at the bottom of a slope close to a river in 2009, and on the top in 2008. This would have resulted in large water contribution from the upslope land after rainfall episodes, causing saturated conditions and even standing water at the soil surface in 2009. Nitrification could have been the cause of the N_2O fluxes during the first days after the first fertilization in both years ('2008I', '2009I'). This is supported by the increase of soil NO_3^--N in all N treatments, especially in PS and CS where the N was applied entirely as NH_4^+-N (Figure 5.3). However, this transformation was slower in '2009I' as soil mineral N was present both as NH_4^+-N and NO_3^--N ten days after fertilization where, at the same stage, mineral N was practically all as NO_3^--N in '2008I'. This was probably caused by the high soil WFPS contents in ˙2009I'. In addition, most of the existing NO_3^--N in MN plots and that already produced in CS and PS and also in MN plots during '2009I' could have been further reduced to N_2 (Smith et al., 2003) at such high soil WFPS levels, explaining the lower N_2O fluxes observed compared to '2008I' (Figure 5.3). The CN plots also had an increase in soil NO_3^--N contents suggesting that mineralization of the soil OM and nitrification had occurred. The increase was larger in 2008 possibly due to the drying and wetting cycles observed during this year. During long periods of dry soil, a large fraction of the microbial population can die, increasing the levels of OM in the soil and, after soil rewetting, the surviving microbial biomass mineralizes that fresh OM (Van Gestel et al., 1993). Also these cycles could disrupt soil aggregates, exposing the large soil OM contents of this soil to a rapid mineralization (Goebel et al., 2005) and consequently emitting large pulses of N_2O, which would be in agreement with other studies carried out in temperate climates (Jørgensen et al., 1998; Kim et al., 2010), semi-arid climates (Barton et al.,

2008; Dick et al., 2001) or even under laboratory incubations (Bergstermann et al., 2011). In our study, the resulting fluxes contributed approximately 40% to the total cumulative N_2O emissions from each treatment and denitrification would have been the largest contributor after both episodes of soil rewetting as soil NO_3^--N contents and WFPS contents were not limiting for losses of N_2O by this pathway (Davidson, 1991).

5.4.2. Impact of mineral or organic fertilizer on cumulative N_2O emissions

Literature reviews including soils planted with maize have reported contradicting emission responses to the application of mineral and organic fertilizers (Table 5.5): greater N_2O emissions from mineral fertilizers in López-Fernández et al. (2007) and Chantigny et al. (2010) in clay soil, lower in Chantigny et al. (2010) in loam soil and van Groenigen et al. (2004). In agreement with our study, similar losses of N_2O from mineral and organic fertilizers are reported in Collins et al. (2011), Cai et al. (2012), Damdreville et al. (2008) and Meijide et al. (2007). The latter experiments, with the exception of those by Cai et al. (2012) and Meijide et al. (2007), corresponded to experimental sites with lower native soil C content but similar soil texture as in our experiment. The application of organic C contained in the slurries to soils with low C contents enhances microbial growth which promotes oxygen consumption and creates temporary anaerobic microsites (Cannavo et al., 2004), enhancing N_2O production by denitrification. Thus, the similar large N_2O emissions observed from mineral and slurry-treated plots in our experiment suggest that sufficient native soil C contents (Table 5.1) were available to support denitrification and that any differences between N_2O emissions were ultimately related to mineral N availability. This is also in agreement with the observations of Chantigny et al. (2010) and Pelster et al. (2012) in soils planted with maize and wheat, respectively, and both had high C contents.

The results reviewed in Table 5.5 confirm that the resulting EFs derived from maize cropping are not always similar the IPCC default value of 1% (IPCC, 2006). Under Mediterranean climatic conditions, even under lower soil C contents, urea or pig slurries under irrigation (López-Fernández et al., 2007; Meijide et al., 2007) resulted in EFs larger than this threshold value. In Canada (Chantigny et al., 2010; Gagnon et al., 2011), when optimal soil moisture conditions for N_2O losses by denitrification are combined with higher soil C contents than those in this study, the resulting EF are even higher. However, in silt loam soils (Collins et al., 2011; Damdreville et al., 2008, Table 5.5) fertilization caused similar or lower losses than 1%, contrary to our study, probably because of the lower soil C availability and greater soil aeration (WFPS 36.1-42.1% in Collins et al. 2011; 45-46%WFPS in Damdreville et al., 2008). This indicates that the IPCC default value understimates N_2O emissions from maize cropping in this region and in this type of soil.

5.4.3. Yield scale N_2O emissions

Expressing N_2O fluxes in terms of crop productivity did not reveal an association between fertilizer type, low N_2O production and high yields, possibly due to the large initial soil N contents in both soil sites. However, the results showed that even when similar types of fertilizers are applied at similar rates in the same soil type, yield scale factors vary largely from one year to other. In this sense, as it was mentioned, N mineralization could probably be more stimulated during 2008 because of the environmental conditions (e.g. soil moisture and temperature, Ma et al., 1999), increasing the amounts of mineral N available in the soil profile compared to in 2009. This resulted in the low efficiency of N fertilizers to increase yields during 2008, leaving larger quantities of N after harvest compared to 2009 (66-425 kg N ha^{-1} in 2008 versus 24-47 kg N ha^{-1} in 2009, data not shown). This indicates that the N inputs from mineralization varied seasonally, in agreement with Zhang et al. (2015) and Loecke et al. (2012) and thus, so do the optimal N rates for maize cropping as N demand changes (Olfs et al., 2005; Raun et al., 2009). So, the N_2O emissions observed during 2008 were related to the N rate that exceeded crop N uptake capacity (Snyder et al., 2009). Nitrogen fertilization strategies to maize cropping in this region must consider possible large N inputs from soil mineralization and adapt N fertilizer rates to match crop demands. Similarly, Hartmann et al. (2014) reported that N applications during maize growing season must be reduced as mineralization meets crop demands and N losses are very likely to occur. Also, they confirm that reductions in N application rates relative to conventional farming practices (excess of N inputs) do not affect maize yields and cause less N surpluses at harvest. Similar conclusions were also reported by Zhang et al. (2015). So, our study highlights the need to increase the knowledge of initial soil N contents at the moment of the N application and the dynamics of soil organic matter mineralization to adapt N rates to efficiently meet crop demands, especially in the period between sowing and top dressing application when demands are small.

Table 5.5. Soil classification and properties (texture, soil OM, C contents), cumulative background and fertilizer induced N_2O emissions, and EF from derived from maize cropping studies reviewed.

							Cumulative N_2O emissions (kg N_2O–N ha^{-1})			Emission factor (%)		
Location	N applied (kg N ha^{-1}) (Control/Treat 1/Treat 2)	Treatments[1] (Control/Treat 1/Treat 2)	Soil classification	Soil texture	C content (g C kg^{-1})	OM (%)	Control	Treat 1	Treat 2	Treat 1	Treat 2	Ref.[2]
Madrid, Spain	0/170/170	control/incorporated pig slurry/ urea	Typic Xerofluvent	Sandy loam	8.2	1.4	2.91	5.08	5.89	1.27	1.80	1
Henan province, China	0/150/150	control/organic manure/NPK	Aquic Inceptisol	Sandy loam	6.46	-	0.06	0.30	0.47	0.15	0.27	2
Quebec, Canada	0/100/100	control/liquid swine manure/mineral fertilizer	clayey, mixed, frigid, Typic Humaquept	Clay	46.8	-	2.63	7.10	11.61	3.1	6.6	3
Quebec, Canada	0/100/100	Control/liquid swine manure/mineral fertilizer	loamy, mixed, frigid, Typic Dystrocryept	Loam	29.9	-	1.44	4.59	2.01	2.4	0.4	3
Wageningen, Netherlands	0/209/188	control/cattle slurry/CAN	Typic Endoaquoll	Sandy	-	4	0.14	1.70	0.25	0.75	0.06	4
Wageningen, Netherlands	0/196/188	control/cattle slurry/CAN	Typic Endoaquept	Heavy clay	-	6.4	1.52	3.37	2.75	0.88	0.66	4
Prosser, USA	0/336/336	control/liquid manure/NPK	coarse-silty, mixed, superactive, mesic Xeric Haplocambid	Silt loam	10	-	0.155	0.411	0.361	0.10	0.08	5
Rennes, France	0/110/180	control/pig slurry/AN	Luvisol Redoxisol	Silt loam	9.5	-	1.00	1.028	2.167	1.07	0.02	6[†]
Rennes, France	0/180/132	control/ matured pig manure/AN	Luvisol Redoxisol	Silt loam	10.1	-	0.344	0.851	0.872	0.38	0.29	6[†]
Madrid, Spain	0/175/175	control/ pig slurry without DCD/ urea	Typic Xerofluvent	Sandy loam	0.0089[††]	1.4	5.98	8.27	8.57	1.30	1.49	7
Quebec, Canada	0/200/200	control/UAN/CAN	fine, mixed, frigid Typic Humaquept	Clay	46	-	6.5	14.2	13.3	3.8	3.4	8

Note: in those experiments where different N rates of the same fertilizer were tested, the resulting N_2O emissions and EF after the application of similar N rates as in this experiment were selected. Also, in those works with more than one year of study testing the same N rate/s of the same fertilizer/s, the mean value of the N_2O emissions and EF obtained was considered.

[1] Treatments: CAN: calcium- ammonium nitrate; AN: ammonium nitrate; UAN: urea-ammonium nitrate; DCD: dicyandiamide

[2] Ref.: 1, López-Fernández et al. (2007); 2, Cai et al. (2012); 3, Chantigny et al. (2010); 4, van Groenigen et al. (2004); 5, Collins et al. (2011); 6, Damdreville et al. (2008); 7, Meijide et al. (2007); 8, Gagnon et al. (2011)

[†] calculated by lineal interpolation of periodic fluxes on the field

[††] expressed in g kg^{-1} of water soluble organic C

5.5. Conclusions

We observed large differences in N_2O losses between the two maize cropping seasons studied even though they were performed in the same soil type. The different location of the sites could have influenced the contrasting soil moisture contents observed during both seasons and the extension of these events, which affected nitrification and denitrification processes. The objective of this study was to identify the N source that produced low N_2O production and high yields. However, we were not able to select one, as the forms of N applied caused similar N_2O losses to produce the same yields, probably due to the high C contents characteristic of the type of soil studied. It was also observed that the large initial soil mineral N contents at both sites, together with the application of the N treatments resulted in an accumulation in the soil after harvesting, especially in 2008. This study highlights the need to increase the knowledge of initial soil N contents at the moment of the N application and the dynamics of soil organic matter mineralization to adapt N rates to efficiently meet crop demands, especially in the period between sowing and top dressing application when demands are small.

CHAPTER 6. SHORT, LONG-TERM GREENHOUSE GAS (GHG) FLUXES AND YIELD SCALE EMISSION FACTORS DERIVED FROM A FORAGE MAIZE SEASON AFTER THE APPLICATION OF MINERAL AND ORGANIC FERTILIZERS IN NW SPAIN

Abstract

The objective of this study was to investigate the contribution of from the different N fertilizers, commonly used in Galicia (NW Spain) for forage maize cropping (*Zea mays L.*), on methane (CH_4), carbon dioxide (CO_2) and nitrous oxide (N_2O) fluxes in a short-term period after their application, to quantify total N_2O fluxes derived from the whole maize grown season and provide yield scaled N_2O emission factors. The treatments applied to a maize field were: (i) control (CN, no added N), (ii) NPK fertilizer + granulated urea (MN),(iii) injected cattle slurry (CS) and (iv) injected pig slurry (PS). Closed chambers were used to monitor CO_2, CH_4 and N_2O fluxes in a period of 9 days (short-term period) after N fertilizers were applied but N_2O fluxes were also measured throughout the whole forage maize season (long-term period). During the short-term period, the largest cumulative Global Warming Potential (GWP) (sum of N_2O, CH_4 and CO_2) values were from PS (0.91 Mg CO_2-C eq ha^{-1}) and CS (0.71 Mg CO_2-C eq ha^{-1}) due to the CH_4 and CO_2 fluxes observed after their injection into the soil. Whereas, MN caused similar cumulative GWP values (0.33 Mg CO_2-C eq ha^{-1}) to a soil with no N fertilizer. However, the cumulative GWP values obtained for PS and CS during the short-term period must be considered carefully as the length of the sampling campaign could have overestimated the real effect of these treatments. In the long-term period, the application of N fertilizers significantly increased cumulative GWP values for N_2O, especially in the period between sowing and top dressing. Global warming potential values for N_2O were 1.56 Mg CO_2-C eq ha^{-1} from CN and between 2.33-2.61 Mg CO_2-C eq ha^{-1} from N-treatments but no differences were observed between N-fertilizer treatments, probably due to the high initial soil mineral N contents at this site and the high mineralization rates observed. As a result, fertilizers did not increase yields, resulting in mean apparent nitrogen recoveries (ANR) of 4.29% (-3.26 to 11.7%) and yield-scaled emission factors of 36.8 g N_2O kg N^{-1} in CN plot and between 53.0-57.9 g N_2O kg N^{-1} in N-fertilized plots.

This study highlights the inefficiency of N fertilizers to increase yields when mineralization is stimulated during maize growing seasons which also resulted in large yield scaled N_2O emissions. Reducing nitrogen application rates in this soil is recommended and total N rates must be adapted to the initial soil mineral N contents. In addition, the large contribution of the soil organic matter mineralization must be considered as a potential pool of N for the crop. Adjusting N fertilizer rates is necessary to avoid excess of N in the soil as it does not significantly increase forage maize yields and it can provide the substrate for large losses of N as N_2O when soil conditions are optimal for both nitrification and denitrification.

6.1. Introduction

Anthropogenic greenhouse gas (GHG) emissions derived from human activities are largely responsible of global climate change. Agriculture contributes to 10-12% of the total global anthropogenic GHG emissions with 60% and 50% of the total global anthropogenic N_2O and CH_4 being generated during agricultural practices (Smith et al., 2007). Following this trend, Spanish agriculture causes 14.1% (40, 218.84 kt CO_2 equivalents) of the country's total GHG emissions, contributing 84.1% and 53.9% to the total anthropogenic emissions of N_2O and CH_4, respectively (MAGRAMA, 2015).

Methane is produced by methanogenic microorganisms under anaerobic conditions (Chan and Parkin, 2001) which takes place during manure storage and when they are applied to water-logged fields (Fangueiro et al., 2008a; Smith et al., 2008). Methane is also consumed by methanotrophes, which are present in many soils (McLain and Martens, 2006). Agricultural soils are considered as sinks of CH_4 as microbial uptake is generally larger than production (Del Grosso et al., 2000). However, some studies have shown that application of N- based fertilizers can reduce the oxidation capacity of soils due to the inhibition of the enzyme responsible of methane oxidation (methane monooxygenase) (Bedard and Knowles, 1989; Knowles and Topp, 1988).

Carbon dioxide emissions can increase after application of organic fertilizers to unamended soils due to the rapid depletion of easily mineralizable carbon by respiring microorganisms as well as to the release of CO_2 dissolved in the organic amendments (Chadwick et al., 2000; Chantigny et al., 2001; Collins et al., 2011; Fangueiro et al., 2008b; Phillips et al., 2009; Rochette et al., 2006; Wulf et al., 2002).

Agricultural N_2O emissions derive from the processes of nitrification (requiring aerobic conditions and N as ammonium (NH_4^+-N)) and denitrification (requiring anaerobic conditions and nitrate (NO_3^--N)). Also, the application of manures provides additional C to soil, which may decrease soil oxygen due to the increase of microbial respiration and generation of anaerobic conditions for denitrification. Thus, N_2O production is usually enhanced after application of N fertilizers especially when N fertilizers are applied beyond crop demand because N forms are available for N_2O production and emission.

The Intergovernmental Panel on Climate Change (IPCC) assumes that 1% of the N applied to soil is lost as N_2O, regardless of type of fertilizer, soil or climatic conditions. However, a large quantity of studies report losses of N_2O larger than 1%. Van Groenigen et al. (2010) argued that agronomic conditions should be included when assessing N_2O emissions. They suggested that expressing N_2O losses as yield-scale N_2O emissions may provide a better understanding of the real impact of the agricultural practices for crop productivity. As Galicia produces 56% of the forage maize in Spain

(2,069,017 Mg, MAGRAMA, (2013)), investigating the environmental impact caused during forage maize production in this region is required. Thus, this paper will be present the data derived from the maize crop season in Galicia during 2010 after mineral and organic fertilization. The objectives of this study were to investigate the contribution of from the different N fertilizers, commonly used in Galicia (NW Spain) for forage maize cropping (*Zea mays L.*), on CH_4, CO_2 and N_2O fluxes in a short-term period after their application, to quantify total N_2O fluxes derived from the whole maize grown season and provide yield scaled N_2O emission factors.

6.2. Material and Methods

6.2.1. Study site and experimental design

The experimental site was located at Mabegondo Agricultural Research Centre (CIAM-INGACAL) (43.2425°N, 8.2608°W) in Galicia, Spain. The climate is European Atlantic (Fernández et al., 2011) and Mediterranean humid according to agrologic classification provided by Papadakis (1966). The annual mean air temperature and total precipitation of the last 10 years (2000-2009) was 13.2 ºC and 1167 mm, respectively. The soil of the experimental site was a Humic Cambisol with a silt loam texture. Soil properties of the upper 10 cm were 21.8% sand content, 57.3% silt content, 14.5% clay content (USDA classification), organic matter (OM) 77.2 g kg^{-1}, total carbon (C) content 31.8 g kg^{-1}, total nitrogen content 2.96 g kg^{-1} and pH 5.3. The soil had a bulk density (BD) of 1.28 Mg m^{-3}. The experimental site was a grassland soil used for grazing during the previous 5 years before this experiment.

The experimental plots were established in May 2010 as a randomized plot design with three replicates of the following treatments: no N fertilization or control (CN), mineral fertilizer as NPK 15:15:15 fertilizer and granular urea 46% (MN), cattle slurry (CS) and pig slurry (PS). The size of the plots were 42 m^2 in area (4.2 m × 10 m) for control and mineral fertilizer plots. To accommodate the 4.4 m application width of the slurry tanker used for the slurry injection, CS and PS plots were 88 m^2 (8.8 m × 10 m). Excluding the CN, all treatments received a target rate of 200 kg N ha^{-1}. Slurry treatments were injected at 20-25 cm depth on 25th May 2010. Mineral fertilizer was applied by hand and split in two doses: 125 kg N ha^{-1} as NPK 15:15:15 fertilizer on 25th May (first application) and 75 kg N ha^{-1} as granular urea 46% on 16th July (second application). After fertilization, plots were seeded to forage maize (Zea mays *L.*) on 28th May at a density of 90,000 seeds ha^{-1}. Row and plant spacing were 75 and 14 cm, respectively. Harvesting occurred on days 28th, 29th and 30th September 2010.

6.2.2. Slurry collection and analyses

Cattle slurry was collected from the pit located at CIAM and the pig slurry was brought from a pit located on a private farm. A sample of each slurry was analysed prior to the fertilization for total N. Based on this analysis, cattle and pig slurries were applied at rates of 90 and 60 m^3 ha^{-1}, respectively, equivalent to 200 kg N ha^{-1}. The methodology to determine the chemical and physical composition of the slurries used (Table 6.1) has been described in Louro et al. (2015).

6.2.3. Plant sampling and analyses

At the optimal forage maize maturity stage, two central lines per plot were selected. In each line, 10 plants in a stretch of 7 m length were cut with a sickle to 20 cm from root formation and weighed and ground. Samples of 300 g were selected for analysis of dry matter (DM) content and crude (CP) and these parameters were used to calculate N uptake in plant and apparent nitrogen recoveries (ANR) as in Louro et al. (2015). Yield scaled N_2O emissions were calculated in each treatment by dividing the total cumulative N_2O fluxes during maize season by the N yields and expressed as and g N_2O-N kg N^{-1}.

6.2.4. Soil sampling and analyses

Soil samples at 10 cm depth were taken once a week for analysis of soil moisture and mineral N contents (NH_4^+-N and NO_3^--N) (methodology described in Louro et al. (2015)). Water-filled pore space (WFPS) was also calculated as in Louro et al. (2015).

Table 6.1. Composition of the slurries applied in 2010.

Properties	Unit	Cattle slurry	Pig slurry
pH		8.3	8.3
Density	g ml^{-1}	1.06	1.01
DM[a]	%	7.66	1.49
NH_4^+-N	%FM[b]	0.099	0.148
NO_3^--N	%FM[b]	0.000	0.000
C	g kg DM^{-1}	229.8	185.4
N	g kg DM^{-1}	37.7	154.3
C/N	-	6.1	1.2
OM[c]	g kg DM^{-1}	780	545
P	g kg DM^{-1}	6.5	21.4
K	g kg DM^{-1}	37.5	131.7
Ca	g kg DM^{-1}	22.7	31.6
Mg	g kg DM^{-1}	6.1	12.6
Na	g kg DM^{-1}	4.6	24

[a] DM: dry matter; [b]FM: fresh matter; [c]OM: organic matter

6.2.5. Greenhouse gas sampling and analyses

Soil CH_4, CO_2 and N_2O fluxes were measured between 9:00 and 10:30 h using the closed chamber technique (Louro et al., 2015). Two polyvinyl chloride (PVC) cylindrical chambers (height: 36 cm; diameter: 25 cm) per plot were inserted between crop rows to a depth of 3cm. On every sampling date, air samples were collected from chamber headspace 60 min after chamber closure. Samples were withdrawn through a rubber septum, located on the chamber lid, by using a polypropylene syringe fitted with a needle and transferred to 10 ml pre-evacuated glass vials. Ambient samples were also taken to provide background values (T_0) for CH_4, CO_2 and N_2O. Air temperature of each chamber was also recorded in each sampling date and the data was used to calculate gas fluxes.

Concentrations of CH_4 and CO_2 were analyzed using a gas chromatograph (GC, Agilent 7890A, Agilent Technologies Inc., USA) fitted with a flame ionization detector (FID) and equipped with a methanizer. Nitrous oxide concentration of the samples was determined by using a Thermo Finnigan

GC 2000 Trace gas chromatograph (Thermo Finnigan, Rodano, Italy) equipped with an electron capture detector.

Fluxes of CO_2, CH_4 and N_2O were calculated using the following equation (Eq.1):

$$F = \rho \times V/A \times (C_{60}-C_0)/t \times (273.15/T) \text{ (Eq.1)}$$

where F is the gas flux (expressed as mg CH_4 m^{-2} h^{-1},mg CO_2-C m^{-2} h^{-1} and µg N_2O-N m^{-2} h^{-1}); ρ the gas density (CH_4: 717 × 10^3 mg m^{-3}; CO_2-C: 0.536 × 10^6 mg m^{-3}; N_2O-N: 1.26 ×10^9 µg m^{-3}) under STP conditions (273 K and 101,325 Pa); V and A are the volume (0.02 m^3) and area (0.0491 m^2) of the chamber, respectively, C_{60} and C_0 (µm^3 m^{-3}) are gas concentrations at chamber closure (T_{60}) minus gas concentration of the ambient samples (T_0), respectively, t is the time of chamber placement (T_{60}) in hours and T is the air temperature, in ºK, at the time of the sampling.

Daily CO_2, CH_4 and N_2O fluxes for each plot (2 chambers) were calculated and the values from the three replicates averaged to obtain the daily fluxes per treatment and the standard error. Fluxes of CH_4, CO_2 and N_2O fluxes were measured during a short-term period, which covered the time immediately after the fertilization (25th May 2010) and the following eight days (until 3rd June 2010). On days 25th and 26th May, chambers were sampled in four occasions, corresponding to the GHG fluxes on 0.06 day (90 min), 0.25 day (6h), 1.04 day (25h) and 1.25 day (29h 30 min) after the application of the fertilizer treatments. On days 2, 3, 6 and 8 in the short-term period, chambers were sampled once a day. Nitrous oxide fluxes observed during the whole maize crop season (25th May-27th September) were considered for the long-term period. Cumulative CH_4, CO_2 and N_2O emissions derived from the short and long-term periods were calculated using the trapezoidal method (Cardenas et al., 2010; Louro et al., 2015). Based on the split of the mineral fertilizer in two doses, cumulative N_2O emissions from the long-term period were studied in two periods: period 'A' (between 25th May and 16th July) and period 'B' (between 16th July and 27th September) (Figure 6.1).

Cumulative CH_4, N_2O emissions resulting from short-period were transformed to CO_2 equivalents based on the Global Warming Potential (GWP) of greenhouse gases relative to CO_2. Radioactive forcing values considered for CH_4 and N_2O were 25 and 298, respectively (IPCC, 2007).

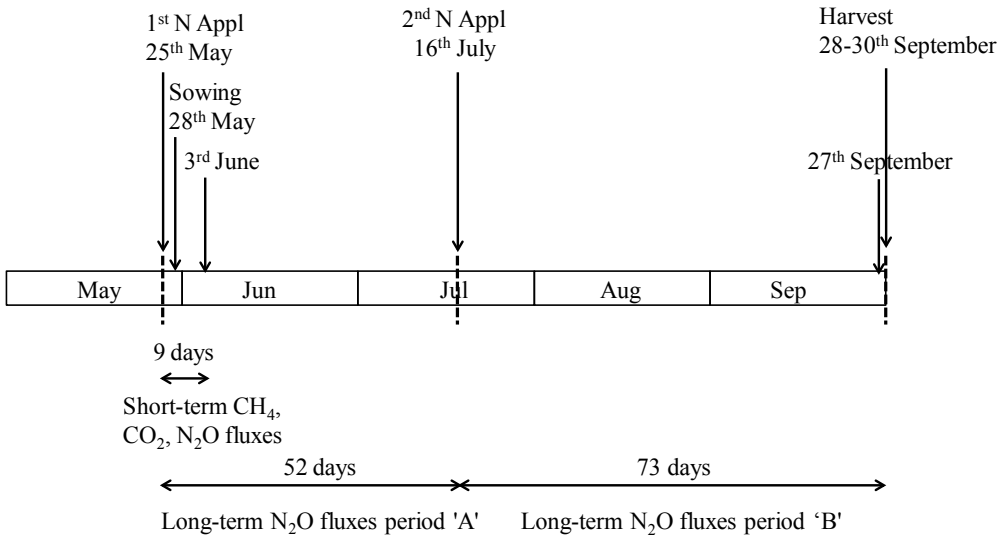

Figure 6.1. Schematic representation of the experiment with the periods considered.

6.2.6. Meteorological data

Total daily rainfall and daily mean soil and air temperatures were recorded between 25th May and 27th September by a weather station located at CIAM. Total annual rainfall in 2010 and mean annual rainfall and air temperature for the last 10 years (2000-2009) were used to compare with the weather conditions observed during the experiment.

6.2.7. Data and statistical analysis

The statistical analyses were performed using Sigmaplot 11. For the hourly N_2O, CO_2 and CH_4 fluxes obtained in the short-term period, two way repeated measures ANOVA was used to test for significant differences between treatments (CN, MN, CS and PS) and between gas sampling events. For the total cumulative CH_4,CO_2 and N_2O fluxes and total GWP resulted from the short-term period monitored, one way ANOVA was used to evaluate the differences between treatments (CN, MN, CS and PS).

For the long-term period, total cumulative N_2O flux data were transformed into mean cumulative fluxes by dividing the total cumulative emissions by the number of days. This allowed comparisons to be made between periods that differed in numbers of days. Two way repeated measures analysis of variance ANOVA was used to determine significant differences between the periods (i.e. period 'A' versus 'B'), including the treatment factor (CN, MN, CS and PS) to determine a significant effect on the mean cumulative N_2O fluxes. For total cumulative N_2O fluxes, one way ANOVA was used to

evaluate the differences between treatments (CN, MN, CS and PS) within each period separately, and also in the overall crop season. For WFPS and mineral N contents, two way repeated measures ANOVA was used to test for significant differences between treatments (CN, MN, CS and PS) and between soil sampling days within each period separately. For DM yield, N uptake, ANR and yield-scale N_2O emissions, one way ANOVA was used to test differences caused by the treatments (CN, MN, CS and PS). In all tests performed Tukey's test was used to determine which treatment groups were different at the $P \leq 0.05$ significance level.

6.3. Results

6.3.1. Weather conditions

During the gas sampling period (25th May-27th September), daily air mean temperatures ranged from 12 to 23 ºC and the soil daily mean temperatures were from 16 to 22 ºC (Figure 6.2a). During the sampling period, a total of 220 mm of rainfall was registered, which was mainly distributed during period 'A' (173 mm) (Figure 6.2a). The important rainfall episodes during period 'A' happened on day 1 (16.7 mm), between days 14 and 17 (115 mm) and on day 45 (19 mm). In the period 'B' the largest rainfall event was observed on day 52 (13 mm).

6.3.2. Water filled pore space (WFPS)

Soil WFPS varied during the experiment (Figure 6.2b). Period 'A' was significantly wetter than the period 'B' due to the largest rainfall events registered in this period. In period 'A' soil WFPS showed values between 54-100% WFPS, whereas in period 'B' values were in the range of 27-60%. No statistical differences between treatments were found within each sampling date ($P>0.05$).

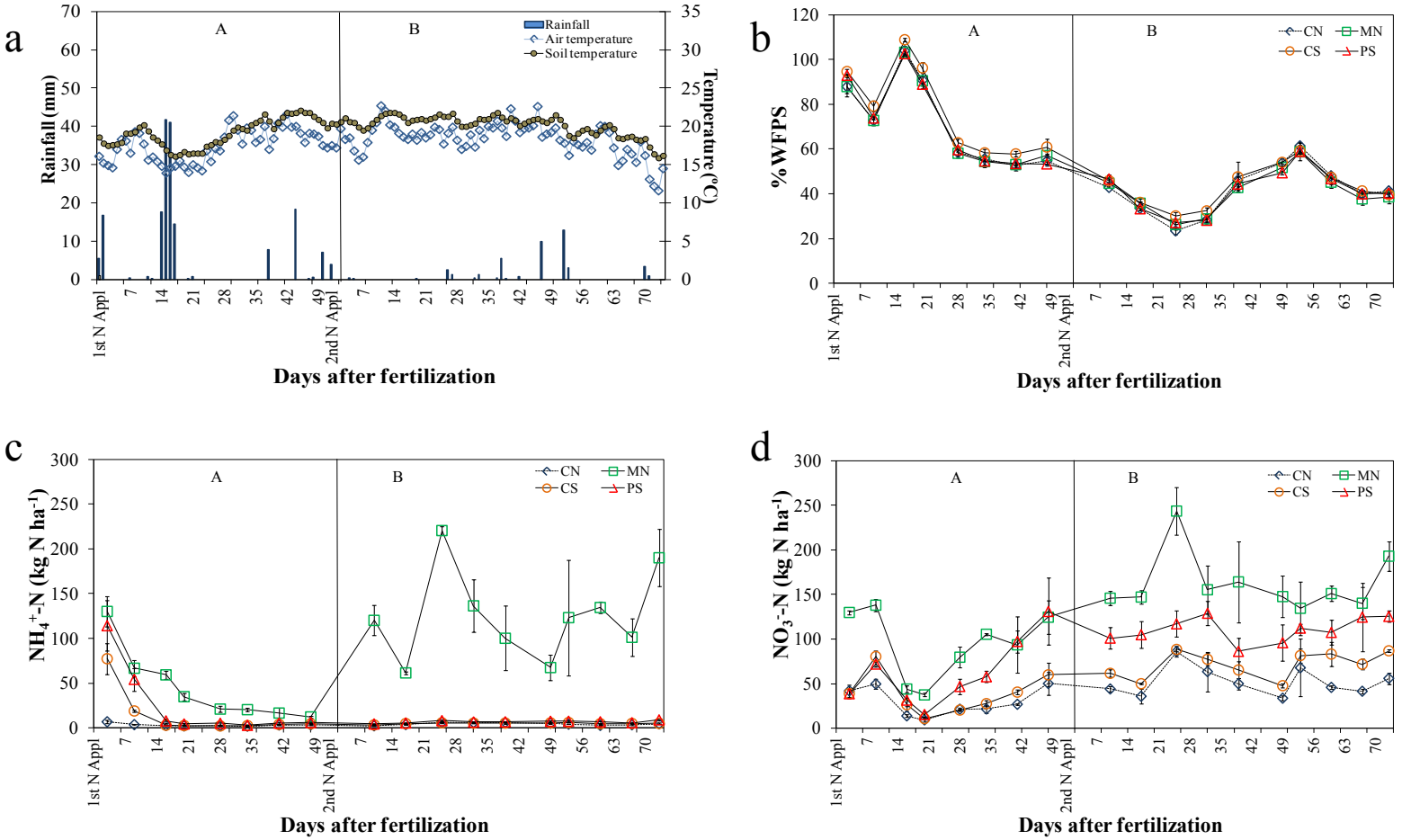

Figure 6.2. a) Daily rainfall, air and soil temperatures, b) %Water filled pore space (WFPS), c) soil NH_4^+-N content and d) NO_3^--N contents during the maize crop season in 2010. Points and error bars represent mean value (n: 3) and standard error, respectively. Letters 'A' and 'B' represent the periods studied. Periods- 'A': 25th May-16th July, 'B': 16th July-27th September. Treatments: injected cattle slurry (CS), injected pig slurry (PS), mineral fertilizer as NPK+ granular urea 46% (MN) and control (CN).

6.3.3. Soil mineral N

6.3.3.1. Ammonium contents

Soil ammonium (NH_4^+-N) contents to 10 cm depth (Figure 6.2c) from CN plots remained below 10 kg N ha^{-1} during the experiment with values in the range 1.9-6.8 kg N ha^{-1}. In the period 'A', maximum soil NH_4^+ maximum contents (10 cm depth) of 130.1, 77.3 and 114.2 kg N ha^{-1} were observed three days after the first N fertilization (1st N Appl) in MN, CS and PS, respectively. After this day, a decrease was observed in all treatments to values of 12.2, 2.2 and 2.9 kg N ha^{-1} in MN, CS and PS, respectively. The statistical analysis showed differences between treatments (P<0.05) and between sampling days (P<0.05). In period 'A' soil NH_4^+-N contents in MN were significantly higher with respect to CS and PS from day 3 to the end of the period 'A' (P<0.05).

In period 'B', the application of the granular urea in MN plots (2nd N Appl) significantly increased soil NH_4^+-N contents in MN plots with respect to the rest of treatments (P<0.05) and these differences were observed until de end of the experiment (P<0.05). Mineral plots produced values from 61.4 to 220.5 kg N ha^{-1} and the maximum value was observed on day 25 after the second fertilization. Plots amended with CS and PS recorded similar values than CN during the period 'B', with ranges from 3.3 to 6.0 and from 4.4 to 9.0 kg N ha^{-1} in CS and PS, respectively.

6.3.3.2. Nitrate contents

Soil nitrate (NO_3^--N) contents (0-10 cm depth) (Figure 6.2d) were in the ranges 9.4-86.0, 10.9-88.4, 15.5-131.0 and 37.4-243.5 kg N ha^{-1} in CN, CS, PS and MN treatments, respectively. The statistical analysis showed differences between treatments and between sampling days (P<0.05).

In period 'A', soil NO_3^--N in CS plots showed similar NO_3^--N levels than in CN plots (P>0.05), contrary to those in PS plots, which were significantly higher (P<0.05). No differences between contents in PS and CS were observed during the first 34 days after the first fertilization (P>0.05). After that NO_3^--N contents in PS plots were similar than MN plots (P<0.05), which was the treatment, in general, with the largest NO_3^--N values. Plots fertilized with MN produced values during period 'A' in the range 37.4 to 138.0 kg N ha^{-1}, larger than those of 9.4-50.2, 10.9-80.4 and 15.5-131.0 in CN, CS and PS, respectively. In period 'B', similar NO_3^--N contents were observed in CN and CS plots, with values in the ranges 33.7-86.0 and 47.8-88.4 kg N ha^- and they were significantly lower (P<0.05) than those observed in MN plots (134.5 -243.5 kg N

ha^{-1}) for all samples taken in this period. Plots fertilized with PS contained NO_3^- -N levels between 86.2 and 129.0 kg N ha^{-1}, which were similar than those in MN during the first 32 days after the second fertilization and similar than in CS plots during the remaining soil samplings.

6.3.4. Short-term effect of fertilization on GHG fluxes

Methane fluxes from CN and MN plots remained negative or close to zero during the short-term period measured after the first N fertilization, with values between -0.06 and 0.05 mg CH_4 m^{-2} h^{-1} in MN and between -0.06 and 0.13 mg CH_4 m^{-2} h^{-1} in CN plots (Figure 6.3a). Fluxes of CH_4 from CS and PS were in the ranges -0.09-5.98 and -0.06-17.70 mg CH_4 m^{-2} h^{-1} from CS and PS, respectively. Plots treated with CS and PS showed significant differences with respect to MN and CN plots at day 0.06 (90 min) (P<0.05), where the largest peaks were observed. Afterwards, peaks of CH_4 from CS and PS rapidly decreased in the next hours to values less than 1 mg CH_4 m^{-2} h^{-1}.

The lowest CO_2 fluxes (Figure 6.3b) were observed in CN and MN plots. Daily CO_2 fluxes were in the ranges -16.3-97.2 and 5.5-63.9 mg CO_2-C m^2 h^{-1} from CN and MN plots, respectively. Plots treated with PS and CS showed significant differences with respect to the rest of treatments within the first 6 hours (on 0.06 and 0.25 days) (P<0.001) with the largest fluxes of 862.3 and 403.0 mg CO_2-C m^2 h^{-1} at day 0.06 (90 min) after the application of PS and CS, respectively. Afterwards CO_2 fluxes from PS and CS decrease to values below 100 mg CO_2-C m^2 h^{-1}, similar than those in CN and MN plots (P>0.05).

The N_2O fluxes from CN and MN plots ranged from 20.6 to 305.1 and from 45.3 to 399.6 μg N_2O-N m^{-2} h^{-1}, respectively (Figure 6.3c). Plots amended with CS and PS produced N_2O fluxes in the ranges 41.2- 492.5 and 46.9-534.7 μg N_2O-N m^{-2} h^{-1}, respectively. Nitrous oxide fluxes from PS showed statistically different N_2O fluxes compared to the rest of the treatments at day 0.25 (6h) and on day 8 after the first fertilization (P<0.05), with the largest flux observed from PS at day 0.25. In the rest of the short-term period measured daily N_2O fluxes were similar between treatments (P>0.05).

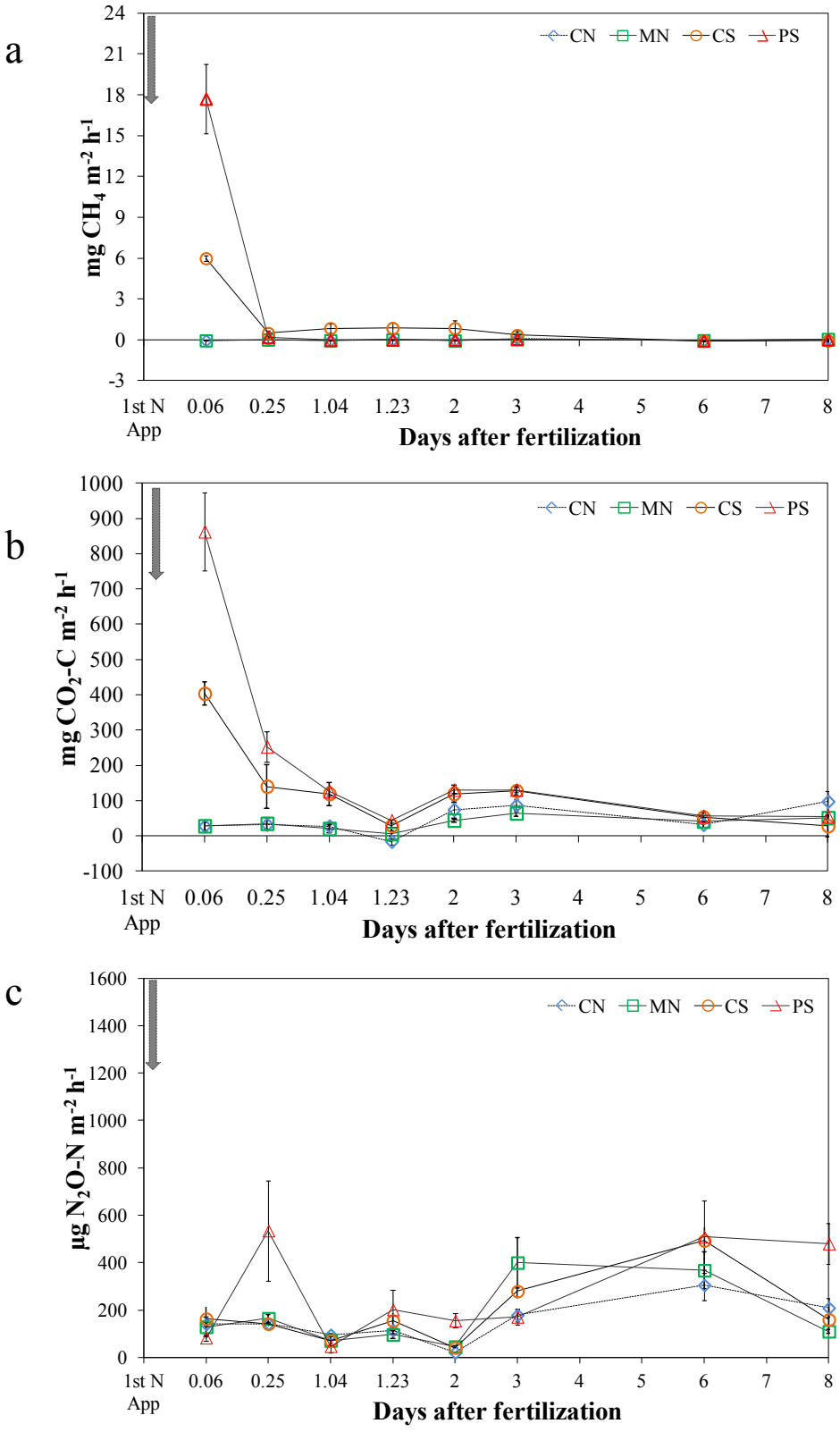

Figure 6.3. Short-term CH_4 (a), CO_2 (b) and N_2O fluxes (c) after the first N-fertilization (25th May) and in the following 8 days. Arrows represent fertilization. Data points represent the mean value of three replicates ± standard error. Treatments: control (CN), mineral fertilizer as NPK+ granular urea 46% (MN), injected cattle slurry (CS) and injected pig slurry (PS). Short-term period: 25th May-3rd June.

6.3.5. Long-term N_2O fluxes

Figure 6.4 presents the field N_2O measurements carried out during 2010 in the whole maize crop season. In period 'A', N_2O fluxes from CN and MN plots showed values from -86.6 to 586.3 and from -124.5 to 502.2 µg N-N_2O m^{-2} h^{-1}, respectively. In CS and PS plots these values were in the ranges -59.0-673.4 and -5.6-645.9 µg N-N_2O m^{-2} h^{-1}, respectively.

In period 'B', N_2O fluxes were in the ranges 26.3-372.5, 24.4 -1052.9, 11.3-988.0 and 35.1-1032.1 µg N-N_2O m^{-2} h^{-1} in CN, MN, CS and PS, respectively. The application of urea in MN plots (period 'B') did not induce an immediate peak of N_2O. The highest peaks observed in this period from N-fertilizer treatments happened on days 53 (in MN) and 54 (in CS and PS). On these days N_2O fluxes of 1052.9, 988.0 and 1032.1 µg N-N_2O m^{-2} h^{-1} were released from MN, CS and PS plots, respectively.

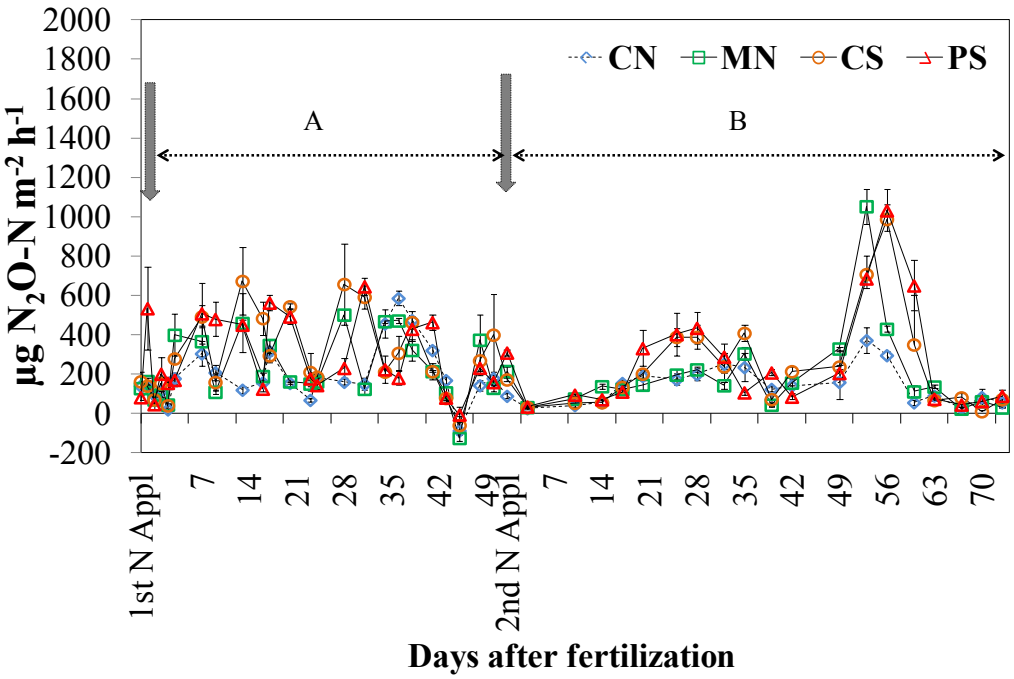

Figure 6.4. Time course of the N_2O fluxes derived from the maize crop season in 2010. Data points represent the mean value of three replicates ± standard error. 'A' 'B' represent the periods studied. Vertical arrows represent fertilizations and horizontal arrows the length of the periods studied. Treatments: control (CN), mineral fertilizer as NPK+ granular urea 46% (MN), injected cattle slurry (CS) and injected pig slurry (PS). Periods- A: 25th May-16th July; B: 16th July-27th September.

6.3.6. Short-term total cumulative GHG fluxes and cumulative global warming potential (GWP)

In the short-term period (Table 6.2), CS and PS showed statistical differences in the total cumulative CH_4 and CO_2 fluxes compared to the CN and MN fertilizer ($P<0.05$) but no differences in N_2O cumulative fluxes were observed during the same period ($P>0.05$). Cumulative CH_4 and CO_2 fluxes from PS were 89% and 24% greater than CS during the short-term period measured. In terms of cumulative GWP potential derived from the first 8 days of measurements (short-tem period), forage maize amended with slurries showed greater values than CN and MN plots ($P<0.05$). Comparing slurry treatments, PS significantly elevated cumulative GWP values with respect to CS ($P<0.05$).

6.3.7. Long-term total N_2O emissions and cumulative global warming potential (GWP)

In the long-term period measured (Table 6.3), total cumulative N_2O fluxes from N-fertilizers (MN, CS, PS) showed statistically differences compared to CN plots ($P<0.05$) but no differences between N-treatments were observed ($P>0.05$) in either period or for the whole crop season. Although, there were differences in the duration of period 'A' and period 'B', cumulative N_2O fluxes were similar in both periods. However, comparing the mean cumulative N_2O fluxes between periods, all treatments in period 'A' resulted in values 1.4-1.7 fold larger compared to those in period 'B'. In terms of cumulative GWP potential for N_2O, N fertilization elevates 1.6-fold compared to no fertilization. (Table 6.3).

Table 6.2. Cumulative CH_4, N_2O and CO_2 fluxes in the first 8 days after the first N fertilization (short-term period) and cumulative warming potential (GWP). Means (standard error) with different letters showing statistical differences (P<0.05). Treatments: injected cattle slurry (CS), injected pig slurry (PS), mineral fertilizer as NPK+ granular urea 46% (MN) and control (CN). Short-term period measured: 25th May-3rd June 2010.

	CH_4 (Kg CH_4 ha^{-1})		N_2O (Kg N_2O-N ha^{-1})		CO_2 (Mg CO_2-C ha^{-1})		GWP
Treat.	Total	Net	Total	Net	Total	Net	Mg CO_2-C eq ha^{-1}
CS	2.250 (0.541)b	2.274 (0.544)b	0.717 (0.030)a	0.192 (0.028)a	0.436 (0.024)b	0.258 (0.050)b	0.71 (0.05)b
PS	4.260 (0.603)a	4.284 (0.613)a	0.887 (0.218)a	0.361 (0.239)a	0.539 (0.024)a	0.361 (0.050)a	0.91 (0.05)a
MN	0.002 (0.015)c	0.026 (0.022)c	0.618 (0.110)a	0.093 (0.124)a	0.149 (0.003)c	-0.029 (0.025)c	0.33 (0.03)c
CN	-0.023 (0.010)c	-	0.525 (0.022)a	-	0.178 (0.027)c	-	0.33 (0.03)c

Table 6.3. Nitrogen applied (kg N ha^{-1}), total (kg N_2O N- ha^{-1}) and mean (kg N-N_2O m^{-2} day^{-1}) cumulative N_2O fluxes in each period and in the whole crop season with cumulative N_2O fluxes transformed to global warming potencial (GWP) (Mg CO_2-C equivalents ha^{-1}). Mean values are presented with standard errors in parenthesis and different letters within each column showing significant differences (P<0.05). Treatments: injected cattle slurry (CS), injected pig slurry (PS), mineral fertilizer as NPK+ granular urea 46% (MN) and control (CN).

	Periods								
	Period A 25th May-16th July			Period B 16th July-27th September			Crop season 25th May-27th September		
Treatment	N applied	Total	Mean	N applied	Total	Mean	N applied	Total	GWP
CS	183	4.46 (0.16)a	0.086 (0.003)a	0	4.31 (0.28)a	0.059 (0.004)a	183	8.76 (0.17)a	2.61a
PS	209	4.31 (0.25)a	0.083 (0.005)a	0	4.34 (0.25)a	0.060 (0.003)a	209	8.66 (0.44)a	2.58a
MN	125	4.29 (0.27)a	0.082 (0.005)a	75	3.55 (0.09)a	0.049 (0.001)a	200	7.83 (0.19)a	2.33a
CN	0	2.73 (0.12)b	0.053 (0.002)b	0	2.51 (0.08)b	0.034 (0.001)b	0	5.24 (0.07)b	1.56b

6.3.8. Crop yield and yield-scaled N_2O emissions

No significant differences between treatments were observed for the parameters DM and N uptake (P>0.05) (Table 6.4). In terms of apparent nitrogen recoveries (ANR), the highest value was observed in CS and the lowest in MN (negative value). However, no significant differences were observed between treatments (P>0.05). Yield scaled emission factors from N-treatments were significantly larger compared to CN (P>0.05) (Table 6.4).

Table 6.4. Dry matter yields, N uptake, N input, ANR and yield scaled N_2O emissions resulting from the whole maize growing season (long-term period). Mean values of three replicates and standard error between brackets. Treatments with different letter within each column and year are significant different (P<0.05). Treatments: injected cattle slurry (CS), injected pig slurry (PS), mineral fertilizer as NPK+ granular urea 46% (MN) and control (CN).

Treatment	Yield	N uptake	N input	ANR	Yield-scale N_2O emissions
	Mg DM ha^{-1}	kg N ha^{-1}	kg ha^{-1}	(%)	g N_2O-N kg $^{-1}$ N
CS	17.4 (1.0)a	166 (8)a	183	10.71 (2.03)a	53.0 (2.6)a
PS	15.6 (1.2)a	151 (13)a	209	3.41 (5.28)a	57.9 (5.1)a
MN	15.0 (0.4)a	140 (4)a	200	-2.10 (4.76)a	56.0 (2.9)a
CN	16.8 (1.0)a	145 (11)a	0	-	36.8 (3.4)b

6.4. Discussion

6.4.1. Fluxes of CH_4 and CO_2 in the short-term period

Methane fluxes were related to slurry applications and were short-lived, with the largest values occurring 90 min after the slurry application. This result is in agreement with other studies, which reported elevated CH_4 emissions within 6 hours (Sherlock et al., 2002), two days (Collins et al., 2011) or even 5 and 7 days after the application of slurries (Jarecki et al., 2008; Sistani et al., 2010), followed by a rapid decline. Contrary to non-slurry treated plots (e.g. MN and CN plots), the low CH_4 fluxes in MN and CN plots indicated consumption also in agreement with Jarecki et al. (2008), Sistani et al. (2010) and Collins et al. (2011), from unfertilized and mineral fertilized maize plots. Based on

the observations reported in many studies (Collins et al., 2011; Sherlock et al., 2002; Wulf et al., 2002), the short-lived CH_4 fluxes after PS and CS application correspond to quantities of CH_4 dissolved in the slurries prior application, probably from the easily decomposable organic fraction through reduction of CO_2 and decarboxylation of acetic acid (Chadwick and Pain, 1997). Based on this, the different magnitude of the CH_4 peaks observed between PS and CS application would be related to the larger fraction of mineralizable C contained in pig slurries (Velthof et al., 2003).

Some studies in forage maize have found that application of N-based fertilizers decreased the CH_4 oxidation capacity of soils. For example, Collins et al. (2011), after excluding the CH_4 fluxes at the time of slurry application, observed that the slurry application lowered the seasonal CH_4 uptake between 55-85% (12 and 37 g CH_4–C ha^{-1}) compared to no fertilization. In this study, we observed that application of MN did not affect soil CH_4 uptake as it resulted in similar cumulative CH_4 emissions than no fertilization. However, in the case of CS and PS, cumulative CH_4 losses were influenced by the fluxes observed immediately their application. If the same procedure as in Collins et al. (2011) was taken into account (i.e. excluding the time of slurry application, 0.06 day), the resulting cumulative CH_4 fluxes did not differ than no fertilization, at least in this short-term period of measurements.

The application of slurries also induced large CO_2 fluxes with respect to soils with no fertilizer as many other studies have reported (Chantigny et al., 2001; Fangueiro et al., 2008b; Rochette et al., 2004). In agreement with these studies the response of the soil to these amendments was short-lived, decreasing after application. In this experiment, this pattern was observed in the first 6h after application, when CO_2 fluxes from PS and CS declined by 71 and 65%, respectively. Reductions of about 50% or larger were observed in Chantigny et al. (2001) in the following 4 h after pig slurry and pig slurry/barley straw applications, in Rochette et al. (2004) and Collins et al. (2011) within 6 h after cattle slurry application to soils planted with maize, and also within a few hours in Fangueiro et al. (2008b) after cattle slurry application to a grassland. Based on the previous works, the large CO_2 fluxes observed immediately after slurry injection would have been caused by the release of the CO_2 dissolved in the slurry itself or from dissociation of the carbonates contained in the slurries when slurries are mixed with the soil. Fangueiro et al. (2008b) did not observe large CO_2 fluxes after application of slurry solid fractions (high DM contents 24.8-26%) as those fractions do not have dissolved CO_2 and water soluble C contents are very low (1.7-2.2g/kg fresh material). In this sense, the differences in the lower DM (Table 6.1) and the larger water soluble C contents (Maag and Vinther, 1999) in the pig slurry could have caused the differences in the resulting CO_2 fluxes immediately after application respect to cattle slurry.

6.4.2. Nitrous oxide fluxes: short and long-term periods

Forage maize in Galicia is commonly grown under weather conditions that ensure even distribution of rain with time once the crop is planted. Nevertheless, the usual rainfall distribution (Louro et al., 2015) was not observed in this study as most of the rainfall fell in a short period of time during the period 'A' instead of more frequent events. This affected the soil WFPS contents during the short and long-term periods studied and, consequently, the resulting N_2O fluxes. A peak of N_2O was observed in PS immediately after its application, probably caused by the release of some N_2O dissolved in the slurry itself. However, the general trend during the growing season was characterized by lower N_2O fluxes during the short-term and long-term periods compared to previous studies in this soil (Louro et al., 2015) even though large soil mineral contents were available (Figure 6.2d). During the short-term period and in the following 13 days, soil WFPS was high, which probably hindered nitrification of the added NH_4+-N with the N-fertilizer. Also, denitrification could have produced N_2 instead of N_2O (Smith et al., 2003) with the NO_3^--N available in the soil. Once most of NH_4+-N was completely transformed in the period 'A' (Figure 6.2c), rainfall decreased and temperatures increased generating more aerobic conditions in the soil which were extended throughout most of period 'B'. (Figure 6.2b). Under these aerobic soil conditions (WFPS<60%, Figure 6.2b), denitrification is limited but not nitrification (Bateman and Baggs, 2005; Davidson, 1991) so the N_2O fluxes observed during most of the dry period would have been caused by nitrification. During the dry conditions in period 'A' the added NH_4^+-N with fertilizers was probably lost as N_2O by nitrification. This would not have been the case during period 'B' as NO_3^-N was the only available mineral N form in PS and CS and the added NH_4^+-N to MN plots during the second application would have not been dissolved due to the lack of rain so organic matter would have been the main source of NH_4^+-N for the losses observed from all treatments during period 'B'. We support this by the fact that CN plots increased the levels of NO_3^--N, which could denote the rapid nitrification of the NH_4^+-N released during organic matter mineralization (Figure 6.2d). We also observed that when rainfall took place at the end of the growing season (days 47 and 53 of the period 'B') soil WFPS increased to optimal values for losses of denitrification (60%WFPS) so that NO_3^--N accumulated in the soil caused the largest N_2O losses observed during the growing season (approx. 1000 mg N_2O-N m^{-2} h^{-1}).

6.4.3. Global warming potential in the short-term experiment and effect of N fertilizers on N_2O emitted during the long-term experiment

Slurry treatments did significantly influence the resulting cumulative global warming potential (GWP) during the first 8 days after application (short-term period) mainly due to the contributions of CH_4 and CO_2 fluxes resulting immediately after application. Sistani et al. (2010) reported the effect of these three gases in short-term (31-33 days) and long-term (141-158 days) periods after application of mineral fertilizer and after injection, surface application and application with soil aeration of a swine effluent during two maize growing seasons. They found in the first year that all N-treatments largely increased GWP during the short period compared to no fertilization but they were similar than no fertilization during the long-term period. In the second year, they observed that injected swine effluent elevated N_2O and CH_4 fluxes during the short-term period, which caused the elevation of the cumulative GWP in the long-term period compared to the rest of treatments. In this experiment, the differences in the GWP from CS and PS in the short-term period with respect to CN and MN were therefore caused by the losses of CH_4 and CO_2 derived from the application of these treatments and not from the soil. Also, as CH_4 and CO_2 were not measured in the long-term period, we cannot know if the influence of these two gases in the GWP during the long term period followed the same trend as in the short-term period. In sight of these, the resulting cumulative GWP in the first 8 days must be considered carefully as the short period of measurements could be overestimating the real effect of the pig and cattle slurry injection on the GWP for the whole maize season, which is also consistent with the findings of Sistani et al. (2010).

6.4.4. Mineral fertilizer versus injected slurries on N_2O fluxes

In agreement with other studies (Chantigny et al., 2010; Pelster et al., 2012) and following a consistent trend observed in previous maize growing seasons soil (Louro et al., 2015), the absence of differences between organic and mineral fertilization was related to the high soil C content of the experimental site. High N_2O fluxes from slurry injection are observed after their application in soils with low C contents because slurries provide C contents that enhance microbial growth. We also observed, based on this study and in the previous maize seasons that the different distribution and quantity of rainfall during maize seasons, and its influence on soil WFPS, can cause differences between 1.5-2.5-fold in the resulting cumulative N_2O losses under the same type of soil, which also concurs with Dobbie and Smith (2003). In terms of the periods studied during the long-term experiment, period 'A' contributed 50% to the resulting GWP despite its shorter length (compared to period 'B') because of the larger proportion of rain that fell in it in combination with the largest quantities of N in the soil, which is consistent with that observed in previous maize seasons in this soil.

6.4.5. Yield scaled N_2O emissions and ANR

The low ANR obtained during this maize growing season denoted the inefficiency of the N fertilizers to increase maize yields. In agreement with Muñoz et al. (2004) and Motavalli et al. (1989), who also obtained low ANR during maize cropping seasons after the application of slurries, the reason of such values is related to the high productivity of the soil. As it was mentioned the soil site was previously a grassland which received large inputs of N during grazing which was reflected in the large mineral N background values once the crop was established (108 kg N ha^{-1} in the 90 cm soil profile, mainly as NO_3^--N, data not shown). In addition, in agreement with Goebel et al. (2005) and with previous maize studies in this soil (Louro et al., 2015), the long period with soil dryness could stimulate mineralization by disrupting soil aggregates, exposing the large quantities of soil OM to be mineralized and further nitrified. That explains the gradual increase of the soil NO_3^- throughout the maize growing season. As the soil OM could have supplied far in excess N crop demands, N fertilizers were inefficient as it was observed in the low ANR values, (Table 6.4), resulting in the accumulation in the 90 cm soil profile of practically all the added N (mainly as NO_3^--N, data not shown). In addition, the fraction of NH_4^+-N in the 90 cm soil profile of MN plots was larger than in CS and PS (127 kg NH_4^+-N ha^{-1} in MN versus 9.24 kg NH_4^+-N observed in both CS and PS plots, data not shown), denoting that the second fertilization in MN plots was not necessary.

The resulting high yield scaled N_2O emissions were reflected in the low ANR, which concurs with the results of the meta-analysis reported by van Groeningen et al. (2010) on published crop studies (maize, wheat potato and rice). However, in their study they reported yield scaled N_2O emissions in the range 8-15 g N_2O-N kg N^{-1}, with the smallest yield scaled emission factors of 8.9 g N_2O-N kg N^{-1} at application rates between 180-190 kg N ha^{-1}, which are far lower than the values obtained in this study or those obtained in previous maize seasons in this type of soil (61.3-122.1 g N_2O-N kg N^{-1}) (Louro et al., 2015). Even in other maize studies, not included in the latter meta-analysis, the resulting values were much lower. In Halvorson et al. (2010), the resulting yield scaled emission factors were in the range of 1.7-3.7 g N_2O-N kg N^{-1} (above-ground N biomass calculated as in van Groeningen et al. (2010)) or between 2.8 and 6.8 g N_2O-N kg N^{-1} in Venterea et al. (2011), both after fertilizer inputs between 0 and 224 kg N ha^{-1}. The differences with our study would be related to the site characteristics (high C, low pH, larger mineralization rates, texture for optimal water retention) and weather-specific conditions, which created optimal soil environment for losses of N_2O. Under these conditions when N additions exceed crop N needs larger losses of N_2O are likely to occur, which is in agreement with McSwiney and Robertson (2005), Snyder et al. (2009) and Snyder et al., (2014).

This study highlights the infectiveness of N fertilizers for increasing yields when mineralization is stimulated during maize growing season. Reducing nitrogen applications rates in this soil is recommended and total N rates must be adapted to the initial soil mineral N contents. In addition, the

large contribution of the soil organic matter mineralization must be considered as a potential pool of N for the crop. Adjusting N fertilizer rates is necessary to avoid excess of N in the soil as it does not significantly increase forage maize yields and it can provide the substrate for large losses of N as N_2O when soil conditions are optimal for both nitrification and denitrification.

6.5. Conclusion

The injection of pig and cattle slurry to a silt loam soil planted to forage maize significantly contributed to global warming potential in a short-term period due to the large CH_4 and CO_2 fluxes released immediately after their application. However, they must be investigated in long-term periods to asses if the contribution of these gases to GWP maintain the same pattern or are overestimated. Nevertheless, short and long-term studies showed that the contribution of the N_2O to GWP was similar between N fertilizers in this soil, with the largest contribution during the period between sowing and top dressing. This study highlights the inefficiency of N fertilizers to increase yields when mineralization is stimulated during maize growing seasons which resulted in large yield scaled N_2O emissions. Reducing nitrogen applications rates in this soil is recommended and total N rates must be adapted to the initial soil mineral N contents. In addition, the large contribution of the soil organic matter mineralization must be considered as a potential pool of N for the crop. Adjusting N fertilizer rates is necessary to avoid excess of N in the soil as it does not significantly increase forage maize yields and it can provides the substrate for large losses of N as N_2O when soil conditions are optimal for both nitrification and denitrification.

CHAPTER 7. GENERAL DISCUSSION AND CONCLUSIONS

7.1. General discussion

The overall aim of this study was to evaluate the impact of the agricultural practices for dairy farming on losses of GHG from soils under the Atlantic climatic conditions in Galicia (NW Spain), in order to make recommendations for practices that reduce emissions and increase crop yields. A series of experiments that address the specific objectives outlined in chapter 1 provide the evidence that agricultural practices in dairy farms in NW Spain significantly increase the risk of losses of N_2O from soils meanwhile largest CH_4 and CO_2 fluxes from soils were short-lived and related to slurry applications. This chapter returns to those objectives and outlines the main findings, limitations and implications. Also, the series of experiments will be used to address the hypotheses raised in this thesis by bringing all the main findings together bringing all the findings together to improve the understanding of losses of GHG from dairy farming in Galicia (NW Spain).

7.1.1. Main findings

The specific objective in Chapter 2 was to develop empirical models to explain inter-annual and seasonal variations of N_2O losses from grazed and fertilized grasslands based on the measurement of the most influential parameters. The experiment in chapter 2 showed that grazing and mineral fertilization significantly increase annual N_2O losses from grassland soils with respect to no N inputs, which was the background N_2O reference of this study. The N_2O data, in addition to soil, weather and N management data, were used to build five empirical models: the first at annual scale and the other four at the seasonal scale (winter, spring, summer and autumn). Four of the five empirical models were significant and explained the variation in N_2O losses. According to the annual empirical model, rainfall distribution, soil WFPS and N management explained the inter-annual variations in cumulative N_2O emissions among 2008 and 2009. However, the variables measured only explained 24% of the inter-annual variations. Inter-seasonal variations in N_2O emitted were also observed but only the empirical models for spring, summer and autumn were significant. In spring, mean soil temperature and fertilizer applications were significant factors whereas in summer the combination of mean soil temperatures and mean soil NO_3^--N contents and in autumn rainfall, mean soil NH_4^+-N, N inputs from grazing and mean WFPS were significant factors.

The second specific objective was to quantify the effects of the application of mineral fertilizers and cattle slurry injection during spring, summer and autumn on GHGs from a grassland with grazing dairy cattle in Galicia (NW Spain) during 2011. In the experiment in chapter 3, Both fertilizers were tested under drier weather conditions with respect to the average, especially after spring and summer applications. The application of mineral and injected slurries under these conditions did not

significantly increase total losses of GHG with respect to a soil with no N-fertilizer applications. A general trend of low N_2O fluxes was observed during the experiment. Soil mineral N was not a limiting factor as, in addition to the added N during fertilizations, organic matter mineralization was also stimulated, increasing the fraction of mineral N in the soil. Thus, N_2O production was only limited when soil WFPS was low. Slurry- treated plots showed positive total cumulative CH_4 fluxes because of the CH_4 release caused during the slurry applications. However, overall, when those fluxes from slurry applications are not considered, negative cumulative CH_4 fluxes were obtained, denoting that, as in mineral fertilized and non-fertilized plots, methanotrophy was the predominate pattern and N additions did not affect the capacity of soil for CH_4 uptake. Carbon dioxide fluxes measured with static chamber represent ecosystem respiration. Short-lived losses of CO_2 resulted from slurry applications, but overall, these large fluxes did not significantly increase cumulative CO_2 fluxes with respect to mineral and non-fertilized plots.

The third specific objective was to investigate how typical management of slurry or mineral fertilizer affect GHG emissions in a grassland soil in the South West of England. As in the previous chapter, in the experiment in chapter 4, mineral fertilizer and cattle slurry were also tested during spring, summer and autumn 2011 to a permanent grassland soil (although with heavier texture than Galician soils). Dry weather conditions were also observed at this site after spring and summer applications, which did result in similar low losses of N_2O from non-fertilized soils. Low soil WFPS during the spring and summer did not favour N_2O production from N fertilizer applications. This fact was attributed to the low mineral N contents of the soil, caused by the large plant N uptake in both N treatments and also due the large fraction of N lost by ammonia (NH_3) volatilization immediately after surface broadcast application of the slurry. After autumn application, losses of N_2O were also low even under wetter soil conditions than in spring and summer. Mineral N levels were also low after the application of the treatments as a consequence of the losses of NH_3 derived from the slurry application and plant N uptake which removed large fraction of the mineral N for N_2O production. However, the N budget from the mineral fertilizer in this period shows a important N surplus, which was not found in the soil and was not lost as N_2O, which could denote the existence of environmental conditions for complete denitrification in autumn. Slurry-treated plots showed positive total cumulative CH_4 fluxes because of the CH_4 release caused by the slurry applications but in the following days the magnitude of the fluxes were similar as in the mineral fertilizer and non-fertilized treatments and losses of CO_2 from both N-treatments were similar than the soil with no N additions, all resulting in a source of CO_2.

As investigated in chapter 5, the forth specific objective was to understand the dynamics of the N_2O fluxes during the maize growing season under the Galician climatic conditions and determine the influence of the type of fertilizer (injected slurries and mineral fertilizers) typically applied by local Galician farmers on the resulting emissions. Investigated chapter 6, the fifth specific objective to investigate the contribution of the different N fertilizers, commonly used in Galicia for forage maize

cropping, to CH_4, CO_2 and N_2O fluxes in a short-term period after their application and quantify total N_2O fluxes derived from the whole maize grown season (long-term period). Consistent results relating to N_2O losses were obtained from both experiments and from the all forage maize cropping seasons: a) using injected slurries (pig or cattle) in a soil with high C contents results in similar cumulative N_2O emissions as mineral fertilizers; b) the largest losses are observed between sowing and top dressing, a period where the wettest conditions are combined with high mineral N levels and low crop demand; c) high initial soil contents of the sites in combination with the dynamic of the organic matter mineralization during the cropping seasons significant contribute to increase mineral N contents in the soil; d) differences in the total cumulative N_2O emissions among cropping seasons were caused by differences in soil WFPS patterns during the cropping seasons and also the existence of dry-wetting episodes, which largely contributed to increase cumulative N_2O emissions; and e) Slurries (pig and cattle) resulted in similar high yield-scaled N_2O emissions as mineral fertilizer, although cattle and pig slurry injection significantly elevated cumulative CH_4 and CO_2 fluxes due to the release of these gases immediately upon their application into the soil but in the rest of days of the short-term period, CH_4 and CO_2 fluxes from soils treated with either pig or cattle slurry did not differ from those treated with mineral fertilizer or no fertilized.

7.1.2. Inter-annual and seasonal variations in N_2O emissions

The first hypothesis was that combining grazing with fertilization under the climatic conditions of the NW Spain can lead in large inter-annual and inter-seasonal N_2O variations from soils, as in other regions of the Atlantic area.

Quantifying annual N_2O losses from different agricultural management scenarios has a important interest for scientists in order to develop strategies to reduce these emissions. However, the task is a real challenge as losses are highly episodic, varying from year to year. One of the important aspects in order to adequately quantify N_2O emissions is the sampling frequency. Closed chamber technique is the most popular option for data acquisition but a frequent sampling campaign is required to reduce errors in the final estimation of the resulting losses (Parkin, 2008; Smith and Dobbie, 2001). In chapter 2, gas samplings were carried out with the highest frequency possible. Only during winter 2008 and autumn 2009, sampling frequency decreased with respect to the general trend and this could explain the absence of significance in the model obtained for winter. Despite this, and considering the general high frequency during the study, the results obtained with this plot scale experiment and consequently, with the resulting models, provide the evidence to accept the tested hypothesis. The inter-annual model was obtained with data from two years, with different seasonal distribution of rainfall among years. That resulted in differences in the soil anaerobic conditions, from very wet

periods to saturation or even with standing water. As denitrification is considered the process that causes the largest losses of N_2O from temperate grassland soils (Saggar et al., 2009), differences in the N_2O/N_2 production ratio caused by the different anaerobic status, could result, in part, in the differences between years. The models also allow some discussion on how emissions may be altered under future climate and farming scenarios.

Martínez de la Torre and Miguez-Macho (2009) modelled a scenario with the future climate changes in Galicia for the period 2034-2051. In this study, a climate change scenario generated by the Atmosphere Ocean Global Circulation model HadCM3 was regionalized by nesting a regional climate model (RAMS) within HadCM3. The regional model grid, centred in Galicia, has a resolution of 25 km which allowed a finer spatial detail of Galicia. According to this study, increases of 1.5°C in the mean annual temperatures and 25% increase in the annual rainfall are predicted for the period 2034-2051 in Galicia. In order to evaluate how the predicted changes in climatic conditions in Galicia for 2034-2051 would affect future N_2O emissions from soils with similar management, the predicted increase in annual rainfall was input in the inter-annual empirical model, considering that soil WFPS would change in the same way. According to this, annual N_2O emissions from soils under similar management would increase by 1.2% during the period 2034-2051.

The inter-annual model also showed that both N fertilization and N excreted also contributed to increase inter-annual N_2O variations. However, in comparison with N fertilizer applications, annual N_2O emissions were more responsive to continuous urine and dung depositions (Saggar et al., 2013), which, according to the inter-seasonal models, contributed to the N_2O emissions during the wettest periods, autumn and winter (although winter model was not significant). In 2009, annual fertilizer rate was reduced by 59% and grazing was practiced throughout the year, increasing annual N excretions by 44% with respect 2008. In this sense, reducing losses of N_2O from animal excreta seems to be an important factor to consider. One option to reduce N_2O emissions could be the use of nitrificator inhibitors as 3,4-dimethyl pyrazole phosphate (DMPP), dicyandiamide (DCD) or and nitrapyrin. These compounds delay the oxidation of the ammonium to nitrate. They act specifically on the enzyme ammonium monooxygenase by blocking the site where ammonium is converted to nitrate, reducing the NO_3^- build up and thus, processes associated with N_2O production. Thus, the application of those compounds to urine patches could significantly reduce these emissions as has been reported elsewhere (de Klein et al., 2011; Di and Cameron, 2012). However, this option could decrease the profitability of the farmers due to their high cost (25-30% of the cost of N fertilizers, Subbarao et al. (2006)). Another way to reduce N_2O production could be the application of restricted grazing plans, which was not applied during the study as livestock remained in the grassland plots until milking time. Restricted grazing plans can be based on the reduction of the number of animals grazing or reducing grazing time in periods where the risk of denitrification is high. After comparing these two options, reduction in grazing time seems to be a more viable option to apply compared to plans aimed

at reducing the number of animals as the latter would decrease farm profitability and make the sector fail to fulfil the high demands of animal protein from the growing human population (Li et al., 2013). By adopting grazing strategies based on reduction in grazing times, significant reductions in N_2O production from soils have been reported in de Klein et al. (2006), Luo et al. (2008) and Schils et al. (2006). Based on observed/modelled data and the ensuing discussion, the development of a grazing plan which restricts access time to grasslands is recommended as first step to reduce N_2O emissions from grazing in this area.

In addition to annual changes in climatic conditions, the models also highlight important changes in the way that temperatures and rainfall and the consequent emissions are distributed throughout the seasons for the period 2034-2051in Galicia (Martínez de la Torre and Miguez-Macho, 2009). The climate predictions show increases in mean temperatures of 1.7°C for spring and 2.5°C for summer compared to the current ones. As the models for these periods have shown, mean soil temperature was the factor that caused the large inter-seasonal variations during spring and summer due to its important effect of stimulating the soil biological processes responsible of N_2O production. In spring, these soil conditions coincide with the period of the peak grassland production so grazing is mainly located during this season. To maintain the grazing intensity, N fertilizers are applied to increase grassland production and provide an available source of N for a very active soil microbial population. In addition, soil OM mineralization was highly dynamic during these periods increasing the fraction of mineral N available in the soil. Under these soil conditions where the microbial population is very active and mineral N was not a limiting factor losses of N_2O were very frequent. This was observed from the grassland plots monitored to obtain the empirical models (chapter 2) but also could explain the losses of N_2O observed from the three forage maize cropping seasons (chapters 5 and 6). Although, differences in soil WFPS between forage maize cropping seasons caused differences in the rate of N_2O produced and thus, the inter-seasonal variation of the N_2O in forage maize, the increase in mean soil temperature during forage maize cropping could also stimulate biological processes for N_2O, which concurs with the observations of Doltra et al. (2015) when modelling data from 2009 and 2010 forage maize seasons (chapters 5 and 6) and data from Danish spring barley cropping system.

The predicted seasonal changes in temperature and rainfall could also have an impact on dry-wetting cycles which this thesis shows to be an important mechanism that increases N_2O emissions from Galician soils. Despite that in chapter 2, the magnitude of these episodes in summer were not as large as those from grazing and fertilization, the effect of dry-wetting cycles were clearly observed during forage maize cropping season in 2008 (chapter 5), causing an increase of 40% in the total N_2O emissions during this cropping season. But also in early autumn 2011 from the grasslands in chapter 3, both following significant rain after long periods of soil dryness. Important changes in rainfall are predicted, in addition to changes in temperatures, for spring and summer seasons in the period 2030-2060 in Galicia (Martínez de la Torre and Miguez-Macho, 2009). Reductions in the rainfall of 25%

are expected in spring and increases of the same magnitude in summer. These extreme changes would lead to more frequent episodes of soil rewetting, which would significantly contribute to increased annual losses of N_2O from soils so more research is required to understand the mechanisms that cause losses of N_2O when these episodes occur in soils in order to obtain potential mitigation options.

7.1.3. Effect of the type of fertilizer on direct losses of N_2O and CO_2 from soils

The second hypothesis was that because Galician soils are generally rich in organic matter content, the addition of slurry-C to soil would not significantly stimulate microbial activity and thus, would not increase CO_2 and N_2O production with respect to increases caused by mineral fertilizer applications as N_2O losses from soils rich in C are more dependent of the availability of N.

Livestock slurries have been used in farms as they are a valuable source of nutrients (N, P, K) for growing crops when used properly. Slurry composition depends on several factors as type of animal, diet and animal age, etc, but in general, when considering the cost of purchasing mineral fertilizers (19% of the total cost for forage production, CAP (2010)), using slurries is a good money saving strategy as well as option to recycle the high volumes of animal wastes generated as consequence of farm intensification. However, there are some environmental concerns about their use as fertilizers. In addition to the odours that emerge after their application (when slurries are not injected) there is the problem related to the increase of direct losses of N_2O from soils compared to mineral fertilizers. Contrary to mineral fertilizers, slurries contain a source of easy mineralizable C which can be used by nitrifiers and denitrifiers to obtain energy. When slurries are applied to soil, microbial respiration increases due to the increase of easily mineralizable C and mineral N contents in the soil. This causes the formation of anoxic microsites within soil, optimal for denitrification and, thus, N_2O production. This causes larger N_2O losses from slurry-treated soils than those with mineral fertilizers (Chantigny et al., 2010; López-Fernández et al., 2007; Rochette et al., 2000b; Velthof et al., 2003). However, according to some studies N_2O emissions differ in rates depending on the type of soil. In low C soils, N_2O emissions are related to easily decomposable organic C whereas in those with high C concentrations where the microbial biomass is limited by other macronutrients, N_2O rates are more often related to N availability (Chantigny et al., 2010; Pelster et al., 2012).

In the experiments described in chapter 3 and 4, cattle slurries were applied to soils with high C contents (48.0 g/kg and 39.4 g/kg, respectively). From the experiment described in chapter 4, it was speculated that the ecosystem respiration resulting from slurry applications was similar to that from non-fertilized and mineral-fertilized soils due to the high C contents of this soil. In the experiment, CO_2 fluxes were measured with static chambers, which accounts for both heterotrophic and autotrophic respiration often referred to as 'ecosystem respiration'. A proxy evaluation of the effect of

slurry C-additions on heterotrophic respiration alone was not possible especially as there were treatment differences in the plant yield which would have affected the proportion of autotrophic relative to ecosystem respiration. In chapter 3, elevated CO_2 fluxes from slurry amended plots immediately after application were attributed to dissolved CO_2 released from the slurry as in other studies (Fangueiro et al., 2008b; Flessa and Beese, 2000; Rochette et al., 2004). Regardless of this short lived increase, the resulting overall ecosystem respiration from soils fertilized with cattle slurry was not significantly different than that from all other treatments. Contrary the results of chapter 4, neither cattle slurry nor mineral fertilizer increased plant biomass relative to no N applications, which would denote that the fraction of CO_2 corresponding to autotrophic respiration was similar among treatments and thus, so was heterotrophic respiration. This means that in soils with high C contents, slurry-C additions do not result in a larger CO_2 response because microbial population obtained enough C substrate from the soil. These results show an absence of a clear overall stimulating effect of slurry- C applications on microbial activity. The lack of microbial stimulation upon organic C addition as slurry would also mean that microbial N_2O producing processes were also not increased by slurry application relative to mineral N application. This result was consistently observed in the overall data for both grasslands (chapters 3 and 4) and forage maize soils (chapters 5 and 6). Even using pig slurry as fertilizer (chapters 5 and 6), which contains a larger fraction of mineralizable C content with respect to cattle slurry (Velthof et al., 2003), similar N_2O responses as cattle slurry or mineral fertilizer were observed. This suggests that the occasional differences in N_2O fluxes caused by organic and inorganic fertilizer types in these soils were more to be due to the existence of available N in the soil rather than C availability (Chantigny et al., 2010; Pelster et al., 2012).

The importance of N availability for losses of N_2O was observed in the English grassland soil in September 2011 (chapter 4), where ammonium nitrate (NH_4NO_3) and slurry (surface broadcast) were applied. In the three weeks following each application, mineral N contents in slurry-treated soils were limited in comparison with those in the soil fertilized with NH_4NO_3. The reason of the limitation was that slurry application rates considered total N contained in the material applied instead total mineral N fraction. In addition, the slurry application technique caused the loss of large fraction of the ammoniacal slurry content by ammonia volatilization (section 7.4), leaving less available N in the soil. However, no larger N_2O loses from NH_4NO_3 were observed after the spring and summer applications as soil WFPS conditions were not optimal for denitrification and plant significantly uptake the mineral N applied. However, under optimal conditions for denitrification observed in autumn, the larger mineral N availability in NH_4NO_3 caused a larger N_2O response in comparison with the slurry treated slurry.

The opposite situation was observed in chapter 3, where slurries were injected into a grassland soil to reduce losses of the available N by NH_3 volatilization (section 7.4). Slurry application target rates aimed to match slurry mineral N contents with those applied with calcium ammonium nitrate (CAN).

Under similar soil mineral N levels, both treatments caused similar N_2O responses in spring and summer. In autumn, where conditions were optimal for denitrification, large N_2O fluxes were observed from both types of fertilizers, although they were significantly larger from soils treated with injected slurry as WFPS levels in soil treated with the organic fertilizer were larger in comparison with those in CAN.

During forage maize, N_2O production was not limited by the availability of mineral N. Prior to fertilization, the soil sites selected for these experiments contained large mineral N contents caused by the previous management of each site. In addition, as it was mentioned in section 7.2, the increase of soil temperatures during the period where forage maize was cropped stimulated microbial activity and thus, OM mineralization, which contributed to increase the mineral N pool in the soil. Under these soil conditions, even when cattle and pig slurries were applied based on the total N contents of the amendments rather than on their mineral N levels, the large fraction of mineral N from the soil compensated differences in mineral N composition between both types of slurry and mineral N fertilizer, resulting in similar N_2O production from all fertilizers tested.

Thus, the results provided with these four experiments provide the evidence to accept the hypothesis that the addition of slurry-C to soils with high C contents does not significantly stimulate microbial activity and thus, increase N_2O production respect to those caused by mineral fertilizer applications as N_2O losses from soils rich in C are more dependent of the availability of N in the soil rather than C. This makes it difficult to choose a specific fertilizer that reduces N_2O and CO_2 emissions so it would be recommended that farmers use organic fertilizers to promote within farm recycling of N.

7.1.4. Slurry application technique and losses of N by NH_3 volatilization

In addition to the hypothesis tested, this data has also contributed to the understanding of how application technique affects emissions. The lower availability of the mineral N when slurries are applied can be caused by the volatilization of the ammonium fraction into the air as ammonia. According to measurements obtained by using a ammonium selective-ion electrode, between 40-48% of the N contained in slurries is as ammonium (D. Baez, personal communication), which is available for crop uptake, and the rest is in an organic form which requires prior mineralization to be available for the crops. This means that maintaining the mineral N form in the soil when slurries are used as fertilizers is crucial for crops. But it is also crucial in order to reduce indirect losses of N_2O associated with NH_3 volatilization, which sometimes can be larger than direct N_2O emissions (Chadwick et al., 2011). To minimize these losses, weather conditions need to be consider. Slurry application is preferred under cool, overcast, calm and misty conditions rather than hot dry weather and windy conditions. In the studies in grassland soils in SW England and Galicia (chapters 3 and 4), slurries

were applied in spring, summer and winter. Except for the autumn application in the Galician soil, the rest of slurry applications were carried out under dry weather conditions, favourable for losses of NH_3. However, irrespective of the weather conditions, the application technique was an influencing factor that determined the degree of exposition of the material to the air and thus, the magnitude of NH_3 losses and the fraction of mineral N available in the soil. In the Galician soil, slurries were applied by shallow injection, which causes less losses of NH_3 than the surface broadcast technique used in the English soil. The ALFAM model (Søgaard et al., 2002), which provides NH_3 estimations and is well correlated to field measurements (Bourdin et al., 2014), was used in the English site to obtained the losses caused by the application technique under those weather conditions. This multiple regression model revealed that 69-86% of the ammoniacal N was lost as NH_3 in the first 24h. No estimations of the resulting NH_3 losses from the slurry shallow injection in the Galician soil were reported in chapter 3. The reason is attributed to the fact that data from Spanish studies were not used to build the ALFAM regression. However, if ALFAM model was used to evaluate these losses under the weather conditions under which slurries where applied in the Galician soil, shallow injection would have caused NH_3 losses between 11-17% of the total ammoniacal N, which is in agreement with the range 1-25% reported by Huijsmans et al. (2001) from field studies. Based on these results it is clear that shallow injection is highly recommended to maintain more fraction of N available plant uptake in the soil and reduce indirect N_2O emissions with respect surface broadcast application technique.

7.1.5. The effect of fertilizer type and applications on CH_4 fluxes from soils

The third hypothesis posed by this thesis was that because of the high fertility of soils in Galicia, additions of N with organic and mineral fertilizers would not reduce soil CH_4 uptake capacity with respect to no fertilization.

Methane fluxes measured were net result of CH_4 production by methanogenesis (positive fluxes) and CH_4 oxidation by methanotrophy (negative fluxes) processes (Baggs et al., 2006; Ball et al., 1999). From the experiments described in chapters 3 and 4, grassland soils result in net CH_4 production when slurries are used as fertilizers. The reason of the net CH_4 production is caused by the large CH_4 fluxes released after their applications which, due to their short-lived nature, were probably caused by the easily decomposable organic fraction through reduction of CO_2 and decarboxylation of acetic acid in the slurry, but not from the soil (Chadwick and Pain, 1997; Collins et al., 2011; Fangueiro et al., 2008b). From the experiments in grasslands, the short-lived CH_4 production attributed to slurry was observed within 2-3 days after its application and in forage maize (chapter 6) over an even a shorter period of time (less than 2h) (chapter 6). Due to the large magnitude of those fluxes, a total positive flux was observed for the whole experiment in both grassland soils, which denoted that

methanogenesis was the most important process in the soils treated with slurries. However, after excluding the time of slurry applications, a total negative flux comparable to that in a non-fertilized soil was obtained for the whole period in both experiments in grasslands, denoting that in temperate grassland soils treated with slurries, methanotrophy is the main process.

Some studies have shown that the N fertilizers can inhibit CH_4 oxidation due to the competition between the NH_4^+-N and CH_4 for methane monooxigenase, the enzyme responsible for the oxidation of CH_4 and others highlight the importance of additions of N for nitrifying population, which also oxidises methane (Bodelier and Laanbroek, 2004). In addition, other studies report inhibitory effects in the CH_4 oxidation capacity of the soil due to the toxic effect of high concentrations of NO_3^--N. From the experiments in chapters 3 and 4 in grasslands, no clear inhibition or stimulation of CH_4 oxidation by the added NH_4^+-N with the slurries or mineral fertilizers was observed nor was inhibition due to NO_3^-N concentrations observed. In the experiment in chapter 4, the reason could be caused by the mineral N levels in the N-fertilized treatments, caused by high plant N uptake and also by volatilization of large fraction of NH_4+-N contained in the slurry. However, in chapter 3, no limitations in soil NH_4+-N levels from N fertilized soils were observed throughout the experiment. Even in summer, the largest mean CH_4 oxidation rates in soils treated with slurry and mineral fertilizer occurred under significant levels of NH_4^+-N (in CS and MN plots) and NO_3^--N (in MN plots) and no differences were observed with the corresponding CH_4 oxidation rates from CN plots. A possible explanation of this result could be in the fact that the soil received N fertilizer applications for many years (Dobbie and Smith, 1996), so the addition of N fertilizers in this study did not have an immediate effect in the resulting oxidation rates. Based on this arguments, farmers would use organic fertilizers to promote within farm recycling of N.

7.1.6. Emission factors

According to the results obtained by Lesschen et al. (2011b) when using the INTEGRATOR model to calculate European N_2O EFs, agricultural soils in the Western part of UK and North West of Spain have medium and high risk of large losses of N_2O, respectively, much larger than those obtained using IPCC default value, due to the large annual rainfall as well as the fine texture of the soils in these regions. The results obtained in this study provide the evidence that EF derived from fertilizer applications to forage crops in the Atlantic area may be lower than 1% proposed by IPCC in the guidelines (IPCC, 2006) under atypical dry conditions, as it was observed in both NW Spain and SW England during 2011. In addition, the magnitude of these EF can be significantly low if dry soil conditions are combined with limited mineral N levels, as it was observed from the English site. On the contrary, when soil moisture and soil mineral N are not limiting factors for N_2O production (Dobbie and Smith, 2003), EFs derived from fertilizer applications can be much higher than 1%, as it

was observed during the three forage maize seasons, where mean EF of 1.64% for mineral fertilizer, 1.63% for injected pig slurry and 1.80% for injected cattle slurry were obtained (chapters 5 and 6). However, as it will be discussed later in this section, the EFs from forage maize were result of an inadequate N fertilizer application rates to soil so optimizing EF derived from forage maize production is required.

Using emission factors based on the rate of N applied does not allow to evaluate the agronomic management as GHG losses depend more than just the amount of fertilized applied. For this reason, the number of studies presented in this thesis aimed to link the agronomic productivity to environmental sustainability. This link is possible by using emission factors based on crop production or as they are also known 'yield-scaled emission factors' introduced by Mosier et al. (2006) and van Groenigen et al. (2010). By using these factors, the most suitable type of fertilization linked with productivity and sustainability would be selected.

In chapter 3, the use of mineral fertilizer was evaluated against using injected cattle slurry as fertilizer in a grassland soil used for dairy grazing. Even though, EF were below the default value of 1% proposed by IPCC, fertilizers did not achieved the main aim of fertilization: increase yields. The dynamic of the OM in the Galician site contributed to an increase in the pool of mineral N in the soil, causing an deficient use of both fertilizers. When expressing GHG losses in DM basis (Mg equiv CO_2-C Mg DM^{-1}) both fertilizers caused similar losses of GHG per unit of crop produced but the resulting factors were only 11% higher than the corresponding value when no fertilizer is applied.

In chapter 4, mineral fertilizer and surface broadcast cattle slurry were also tested in a permanent grassland. Although yield-scaled emission factors were not directly reported, using mineral fertilizers reported slightly larger losses of GHG per unit of yield produced than surface broadcast slurry (P=0.486) due to the larger N availability in the mineral fertilized soil compared to the slurry-treated soil. When the resulting ANR values obtained for the total N applied with each treatment were evaluated, ANR from mineral fertilizers were much higher than the corresponding for slurry application. However, it has to be considered that losses by NH_3 volatilization were very important, reducing the quantity of N in the slurry-treated soil. Thus, if those losses caused by the slurry application technique were considered and discounted to the total N applied, 42.4% of the N finally applied to the soil (70 kg N ha^{-1}) was harvested, which is not very different than the 57.1% obtained after application of 240 kg N ha^{-1} with mineral fertilizer. This means that, in proportion, cattle slurry was able to produce significant yields with less quantities of N.

Based on these results, the author, in principle, recommends the use of slurries as fertilizers in grasslands in the Atlantic area but using the shallow injection technique to reduce losses of NH_3 and increase the fraction of mineral N available for plant uptake. This fertilizer option will reduce the cost

of milk production by reducing the cost of purchasing mineral fertilizers as well as recycle animal wastes produced in farms.

In relation to maize cropping (chapters 5 and 6), the agronomic management only could be evaluated based on losses of N_2O, as CH_4 and CO_2 were only measured in 2010 and in a short-period of time, so future studies in forage maize must include measurements of CH_4 and CO_2 during the whole cropping season. Despite all types of fertilizer resulting in similar N_2O emission per yield, no recommendation can be make in terms of type of fertilizer as the N application rate selected (200 kg N ha^{-1}) was very high and did not only not increase N uptake but also provided the source of N to increase losses of N_2O when soil conditions were optimal. These results concur with the meta-analysis reported by Van Groenigen et al. (2010) on published crop studies (maize, wheat potato and rice) who found that yield-scaled N_2O emissions are negatively related with the N use efficiency so the strategy to reduce N_2O should be focus on optimizing the efficiency of the N fertilizers. The reason of the inefficiency of the fertilizers applied was in the fact that the initial soil N contents and the high source of N from the mineralization of the residues of previous crops (i.e. pea/triticale in 2008 and 2009 and grassland in 2010) were dismissed at the time of fertilizer applications. As García-Pomar et al. (2012) stated based on the results obtained from the agronomic N balance for forage maize cropping in seven Galician dairy farms in 2008 and 2010, both factors are crucial in order to carry out an optimal fertilization. In terms of the initial soil N contents, the latter study pointed that in some sites N fertilization were not required as the initial soil mineral N contents were very high. They also reported that reductions of up to 125 kg N ha^{-1} in the quantity of N fertilizer applied are possible when the previous crops to forage maize are legumes or grasslands because the organic matter provided by previous crops can be mineralized and available for the forage maize. Based on this, N application rates need to be adapted to the initial soil contents and the dynamics of the soil organic matter mineralization need to be studied to finally match N crop demands. Determining the initial soil mineral N contents prior any N fertilizer addition by taking soil samples provides initial information of how much N is available in the soil. However, the estimation of the fraction of mineralizable N that can be available for forage maize uptake during the growing season is difficult due to the variations in the N cycle caused by variations in moisture and temperature, as it was observed in the mineral N pattern in the plots used as control with forage maize. In this sense, scientists in this region need to develop a soil test to predict N supply from organic matter mineralization. For sites like in this study with high dynamic of the mineralization and low crop response to N fertilizer applications, the Illinois soil nitrogen test (ISNT), developed by Khan et al. (2001) would be an appropriate test to consider. This test estimates the amino sugar-N fraction in the soil and quantitatively measures NH_4^+-N levels and it has aided the provision of N recommendations in maize cropping in USA as Williams et al. (2007), Mulvaney et al. (2001), Klapwyk and Ketterings (2006) and Lawrence et al. (2009) have reported.

7.2. Future research

The discussion highlighted a number of areas where future research should focus and an improved understanding in those areas is required if mitigation measures for dairy agriculture in the NW Spain are to be identified and effectively implemented. Those areas are:

- Development of long-term studies based on frequent N_2O measurements as well as the measurement of a larger number of soil and field management variables in order to obtain empirical models that fully explain the inter-annual variations in the N_2O emissions from Galician grassland soils.

- Evaluate if development of a grazing plan based on restricted access time to grasslands would decrease N_2O production from grassland soils in this region with respect to grazing with unlimited access.

- Investigate the processes that trigger N_2O production from Galician soils after dry-wetting periods and develop measure to address this.

- Develop a soil test for Galician soils to predict N supply from organic matter mineralization in order to adapt fertilization rates for forage maize cropping.

- Monitor, in addition to N_2O, CH_4 and CO_2 fluxes from the entire forage maize growing season and in soils with different initial mineral N contents, in order to aid identification of the fertilizer type that gives high yields and low GHG emissions.

- Development of long-term studies that include measurements of soil C sequestration.

- Laboratory studies to evaluate in more detail the effect of slurry-C additions on heterotrophic respiration in Galician agricultural soils with high C contents.

- Laboratory experiments in order to investigate the contribution of nitrification and denitrification processes to N_2O production in Galician soils under different regimes of soil WFPS, specially N_2O/N_2 production ratios by denitrification under those regimes.

7.3. Conclusions

1. Large inter-annual and seasonal variations in the N_2O emissions from grasslands with dairy cattle grazing management and mineral fertilization were observed. These losses were driven by climatic and soil variables but also affected by differences in N management. Under a scenario of future climate change in Galicia, predicted by a global climatic model, better grazing management could help to reduce annual N_2O losses derived from N-management. Dry-wetting episodes can significantly contribute to increased annual N_2O emissions from soils and therefore justifies further research to indentify the exact mechanisms in Galician soils.

2. In grasslands, the use of cattle slurry as fertilizer did not significantly increase N_2O emissions with respect to mineral fertilization. Similar conclusions were obtained during forage maize cropping when organic fertilizers (injected cattle and pig slurries) were compared with mineral fertilization. The soils used in these experiments were C-rich so this factor did not limit N_2O production, and the large soil mineral N contents stimulated the losses of this gas.

3. Modelling the effect of slurry application technique on emissions from grasslands showed that large proportions of mineral N are lost from slurries by ammonia volatilization when surface broadcast compared to when injected. When using slurries as fertilizers, shallow injection is recommended rather than surface broadcast application in order to mitigate indirect losses of N_2O and increase the fraction of mineral N available for plant N uptake.

4. In terms of emission factors, the fraction of N lost as N_2O was lower than 1% proposed by IPCC when fertilizer applications coincided with dry weather conditions and mineral N was limited in soils. However, under the typical climatic conditions of the Atlantic area, that led optimal WFPS values for denitrification, losses of N_2O derived from fertilization can reach values beyond 1%, especially when soil mineral N levels are large.

5. Grassland soils in the Atlantic area were sinks of CH_4 and mineral fertilizer applications did not modify soil capacity to CH_4 uptake. Similar conclusions were obtained for slurry applications, if the CH_4 emissions observed immediately after slurry application that resulted from the release of the dissolved CH_4 in the slurry were not considered. That denoted that the N applications to soils that

received N for many years did not modify methanotrophy activity of the microorganisms present in those soils.

6. Slurry applications did not cause an overall effect in the ecosystem respiration compared to non-treated or mineral fertilized grassland soils even when the resulting high CO_2 emissions observed immediately after slurry applications were considered. In grassland soils, mineral fertilizers and cattle slurries caused similar total CO_2 equivalents to produce the same yields, so both fertilizers could be used. However, if the costs of purchasing mineral fertilizers is considered, using injected slurries as fertilizer would be more beneficial for dairy farmers as animal wastes produced on farms would be recycled and milk production costs would be reduced.

7. In forage maize soils, organic and mineral fertilizers resulted in similar yield-scaled N_2O emissions (expressed as dry matter or N uptake) . However, they failed to efficiently increase crop yields and caused high losses of N_2O. Thus, the initial soil N contents at the moment of the N application and the dynamics of soil organic matter mineralization must be considered to adapt N rates to efficiently meet crop demands, especially in the period between sowing and top dressing application when demands are small.

7.4. Conclusiones

1. Se observaron grandes variaciones interanuales y estacionales en las emisiones de N_2O procedentes de praderas fertilizadas con fertilizante mineral y aprovechadas por pastoreo con ganado vacuno de leche. Estas pérdidas estuvieron condicionadas por las variables climáticas y de suelo, pero también por las diferencias en el manejo del N. Bajo un escenario de futuro cambio climático en Galicia, previsto por un modelo climático global, una mejora en la gestión del pastoreo podría ayudar a reducir las pérdidas anuales de N_2O derivadas de esta práctica. Los episodios de sequía seguidos de precipitaciones que humedecen el suelo pueden contribuir de manera significativa a incrementar las emisiones anuales de N_2O de los suelos y, por lo tanto, este hecho justifica seguir investigando en la identificación de los mecanismos que producen las emisiones del gas en los suelos gallegos.

2. En praderas, la aplicación de purín de vacuno como fertilizante no aumentó de forma significativa las emisiones de N_2O con respecto a la fertilización mineral. Conclusiones similares se obtuvieron durante el cultivo de maíz forrajero cuando se compararon fertilizantes orgánicos (purín de vacuno y porcino inyectados) con la fertilización mineral. Los suelos utilizados en los experimentos eran ricos

en carbono por lo que este factor no limitó las emisiones de N_2O, y los elevados contenidos de N mineral en suelo favorecieron las pérdidas de este gas.

3. La modelización del efecto de la técnica de aplicación de purines en praderas mostró que una gran proporción del N mineral del purín se pierde por volatilización del amoníaco cuando el purín se distribuye en superficie en comparación con la inyección. Cuando se aplican purines como fertilizantes, la inyección superficial es recomendable en lugar de la distribución en superficie con el fin de mitigar las pérdidas indirectas de N_2O y aumentar la fracción de N mineral disponible para la planta.

4. En cuanto a los factores de emisión, la fracción de N perdido en forma de N_2O fue inferior al 1% propuesto por el IPCC cuando la aplicación de fertilizantes se llevó a cabo en condiciones de sequía y cuando el N mineral en suelo fue factor limitante. Sin embargo, en condiciones climáticas más típicas de la zona Atlántica, que propiciaron valores de WFPS óptimos para la desnitrificación, las pérdidas de N_2O alcanzaron valores superiores al 1%, viéndose especialmente favorecidas por las elevadas cantidades de N mineral en suelo.

5. Los suelos de praderas en la zona Atlántica actuaron como sumideros de CH_4 y las aplicaciones de fertilizantes minerales no modificaron la capacidad del suelo para la captación de CH_4. Conclusiones similares se obtuvieron con la aplicación de purines si no se consideran las emisiones de CH_4 producidas inmediatamente tras la aplicación y que fueron atribuidas a la liberación del CH_4 disuelto en el purín. Esto denota que las aplicaciones de N en suelos que recibieron N durante años no modifican la actividad metanotrófica de los microorganismos presentes en dichos suelos.

6. La aplicación de purines no causó un efecto general en la respiración del ecosistema en comparación con los suelos de pradera no fertilizados o con aplicación de fertilizante mineral, incluso cuando se consideraron las elevadas emisiones de CO_2 producidas inmediatamente después de la aplicación del purín. En los suelos de praderas, fertilizantes minerales y purines generaron similares emisiones equivalentes de CO_2 para la obtención de rendimientos de cosechas semejantes, por lo que ambos fertilizantes podrían ser recomendados. Sin embargo, si se considera el coste de la compra de fertilizantes, el uso de purines inyectados como fertilizante sería más beneficioso para los productores de leche ya que las deyecciones generadas en las granjas se reciclarían y los costes de producción de leche se reducirían.

7. En suelos con maíz forrajero, fertilizantes orgánicos y minerales también propiciaron emisiones similares de N_2O para la obtención de los mismos rendimientos de cultivo (expresado por unidad de materia seca o N extraído). Sin embargo, no lograron aumentar de manera eficiente los rendimientos del cultivo y causaron grandes pérdidas de N_2O. Por ello es necesario adaptar las dosis de N a las demandas del cultivo, considerando el contenido inicial de N mineral en el suelo en el momento de la

aplicación del N así como la dinámica de la mineralización de la materia orgánica del suelo, especialmente en el período entre la siembra y la aplicación de cobertera donde la demanda del N por el cultivo es menor.

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APPENDIX 1

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Effect of slurry and ammonium nitrate application on greenhouse gas fluxes of a grassland soil under atypical South West England weather conditions

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Plant N offtake

ABSTRACT

In this study we evaluated how typical split applications of cattle slurry (SL) or mineral fertilizer (AN) (in spring, summer and autumn) affected greenhouse gas (GHG) emissions from a grassland soil. Field measurements were carried out between May and November 2011 using the closed chamber technique. The experiment was located in the South West of England, an area which is typically characterized by high annual precipitation (>1000 mm year^{-1}) and cool temperatures (average annual air temperature of 9.6 °C). The unusual dry climatic conditions observed during the late spring and summer, and the rainfall events identified in autumn affected soil water filled pore space (WFPS) resulting in low nitrous oxide (N_2O) fluxes during the experiment. After the first two applications, climatic conditions dried the soil to values below 60%WFPS, the threshold level for losses of N_2O by nitrification. In contrast, the frequent rainfall events observed after the third application (in autumn) increased the WFPS and promoted losses of N_2O by denitrification. In terms of fertilizer type, AN resulted in higher cumulative N_2O emissions compared with SL after the third application, probably because the SL treatment resulted in more anaerobic soil conditions and ammonia (NH_3) volatilization resulted in a smaller mineral N pool in the soil available for N_2O production and emission. Ammonia (NH_3) emission modelling estimated losses of N by volatilization of NH_3 between 25% and 38% of N applied after slurry surface broadcast application. Plant N offtake represented nearly all of the total N applied in AN plots following the first two applications and 59% of that applied in the third, whereas in SL plots an average of 64% of the total N applied in the three applications was harvested in the grass. Nitrogen gas (N_2) fluxes were not measured but the large rainfall events observed after the third application gradually increased the soil WFPS to saturation and could also have resulted in losses of N by complete denitrification, especially from the AN treatment. Thus, applications of AN and SL resulted in total N_2O-N losses during the 6-month measurement period of 0.21 and 0.17 kg N ha^{-1}, respectively (representing only 0.02% and 0.003% of the N applied). Methane (CH_4) production was observed in the first two or three days after SL spreading. For the remaining days, and also in plots treated with AN, the soil acted as a sink of CH_4 (consumption). Total net CH_4 cumulative values of −0.09 and 0.92 kg CH_4 ha^{-1} were observed in AN and SL, respectively. CH_4 consumption and production rates were related to changes in the %WFPS. Thus, dry soil conditions (below 60%WFPS) enhanced the CH_4 consumption in AN plots and reduced the rate of CH_4 production in SL plots during May and June. Total net cumulative carbon dioxide (CO_2) fluxes of 1.24 and 0.35 Mg CO_2-C ha^{-1} were observed in AN and SL plots during the 6-months measurements.

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1. Introduction

Agriculture and agricultural production practices play an important role in the global fluxes of the greenhouse gases (GHG) methane (CH_4), nitrous oxide (N_2O) and carbon dioxide (CO_2), each contributing 6.5%, 5.5% and 0.1% to the total anthropogenic greenhouse gas emissions, respectively (Sanger et al., 2011). The production or consumption of these gases is mainly due to biological processes which are strongly affected by natural conditions and agricultural management (Snyder et al., 2009). Weather conditions and the addition of slurries or mineral fertilizers (such as ammonium nitrate (NH_4NO_3)) can significantly affect the interchange of greenhouse gases between the soil and the atmosphere. This is due to an increase in the supply of substrates and a change in soil conditions that alters the processes responsible of the production and/or consumption of each gas. Nitrification and/or denitrification can produce N_2O emissions. Both processes occur if nitrogen (N) is applied in the ammonium (NH_4^+-N) form, but only by denitrification if it is applied solely in the nitrate (NO_3^--N) form (Clayton et al., 1997) or after NH_4^+-N has been converted to NO_3^--N. Nitrification is responsible for the release of N_2O in dry or well-aerated soils (as it is an aerobic process), while denitrification is, dominantly, the main pathway at medium–high soil water contents (being an anaerobic processes). The production of CH_4 is primarily by microbial degradation of organic matter under anaerobic conditions, e.g., in wetlands or from rice paddies. However, the application of animal manures can provide an immediately available carbon and moisture source leading to CH_4 production (Chadwick and Pain, 1997). Well-aerated upland soils (e.g., grasslands, forest and arable) are commonly regarded as a biological sink of atmospheric CH_4 and are responsible for 6% of the global methane consumption (Le Mer and Roger, 2001). Carbon dioxide (CO_2) emissions resulting from respiration in soil and by vegetation are the principal sources of CO_2 entering the atmosphere (Smith et al., 2003). Applications of livestock slurries and mineral fertilizers provide nutrients for plant and soil microbial growth, and enhance CO_2 emissions, although N fertilizer effects on soil CO_2 emissions are dependent on weather conditions during the growing season (Morell et al., 2011). Soil moisture influences gas exchange by altering oxygen availability and gas diffusivity. Soil respiration has been shown to increase with increasing temperature due to the enhanced microbial activity. Because of varying weather and soil conditions, the GHG fluxes are likely to be temporally dynamic and also dependent on the nature of fertilizer inputs.

The main aim of this study was to investigate how typical management of slurry or mineral fertilizer affect GHG emissions in a grassland soil in the South West of England. We applied cattle slurry and NH_4NO_3 to grassland plots on three occasions over a period of six months. The fluxes of N_2O, CH_4 and CO_2 were measured at high frequency using the static chamber methodology. Although unplanned, the experiment was carried out under atypical, i.e., unusually dry, climatic conditions for the region.

2. Materials and methods

2.1. Location

The field trial was carried out in permanent grassland in May 2011 at Rothamsted Research, North Wyke, Devon, UK (50:46:10N, 3:54:05W), which has a temperate maritime climate (Koppen, 1931), typical of the South-West England. The 40-year mean annual temperature (1961–2000) is 9.6 °C, and the minimum and maximum monthly mean temperatures are 4.5 °C in February and 15.5 °C in August. The mean annual precipitation (40-years average) is 1056 mm, 46% of which falls between October and January.

Table 1
Chemical and physical properties of the cattle slurry used on each application.

Properties	Units	Applications 1st (16th May)	2nd (24th June)	3rd (8th September)
Density	kg l^{-1}	0.995	1.021	1.006
pH	–	6.9	6.8	7.3
Dry matter	%	8.8	6.4	6.5
Total C	% DM[a]	37.6	39.4	38.4
Total N	% FWt[b]	0.36	0.35	0.32
Total N	% DM	2.58	2.67	2.67
NH_4-N	% FWt	0.16	0.12	0.12
NO_3-N	% FWt	0.00	0.00	0.00
LOI	% DM	74.8	77.7	77.1
Ash	% DM	25.2	22.3	22.9
P as P_2O_5	g l^{-1} FWt	1.11	1.05	1.03
K as K_2O	g l^{-1} FWt	2.99	2.82	2.89
Ca as CaO	g l^{-1} FWt	5.45	3.10	2.37
Mg as MgO	g l^{-1} FWt	0.72	0.74	0.74
S as SO_3	g l^{-1} FWt	0.86	0.79	0.79

[a] DM: dry matter.
[b] FWt: fresh weight.

British soil classification (Avery, 1980) defines the soil as clayey typical non-calcareous pelosol of the Halstow series and as a stagnivertic cambisol, and as aeric haplaquept using FAO and USDA taxonomy, respectively. The soil texture was a silty clay loam (Harrod and Hogan, 1981). Initial analysis of the properties of the upper 10 cm of the soil profile indicated a total N content of 0.44%, total carbon (C) content of 3.94%, C:N ratio of 8.9, pH of 5.9 and bulk density (BD) of 0.88 Mg m^{-3}.

2.2. Experimental design

The experiment was set up in a randomized block design, with three replicate plots of the following treatments: (1) control with no N application (zero N), (2) mineral fertilizer as NH_4NO_3 (AN) and (3) cattle slurry (SL). Replicate plots were 15.0 m^2 in area (3.0 m × 5.0 m), and within each main replicate plot three zones were marked out separating chamber, soil sampling and grass yield measurement areas. Within each plot, three chambers were fixed within an area of 3.0 m^2 (3.0 m × 1.0 m), whilst two zones each of 6.0 m^2 in area (3.0 m × 2.0 m) were left for the soil sampling and grass yield measurements. A distance of 1 m was kept between plots.

2.3. Application events and characteristics of mineral fertilizer and slurry used

The applied NH_4NO_3 comprised 34.5% N. Cattle slurry was collected from a dairy farm located near North Wyke. Treatments were applied by hand three times during the experiment (1st application: 16th May, 2nd application: 24th June and 3rd application: 8th September) at a target rate of 80 kg N ha^{-1} (8 g N m^{-2}) on each application. Slurry was analyzed for total N prior to each application (Table 1). Slurry was spread at rates of 2.26 l m^{-2} in May and June and 2.48 l m^{-2} in September, and the NH_4NO_3 at the rates of 23.2 g m^{-2}, equivalent to 80 kg total N ha^{-1} at each of the three applications.

2.4. Chamber design and operation

The closed chamber technique was used to quantify the GHG flux measurements in the field (Rochette and Erisksen-Hamel, 2008). Chambers comprised white polyvinyl chloride (PVC) open ended boxes with a volume of 0.032 m^3 (length 40 cm, width 40 cm, height 30 cm, Cardenas et al., 2010). The upper edge of the chamber

had a 'U' shaped channel, which was filled with water to ensure an airtight seal with the PVC lid. The lid was fitted with a sampling port with a three-way valve. Three chambers were used per replicate plot (i.e., 27 chambers in total). To ensure a good seal between the chamber and soil, the chambers were inserted into the soil to a depth of 10 cm > 24 h before the flux measurements began (Parkin and Venterea, 2010) and left in the same place until the first plot harvest to avoid soil disturbances, which could affect the soil-atmosphere gas transfer. Chambers were returned to the same position after each plot harvest. The effective height of each chamber above the ground (H) was measured three times: at the beginning of experiment (5th May) and after the first and second grass harvests (22nd June and 24th August). Height measurements were taken internally at the center of each wall and in the center of the chamber. The resultant chamber effective height was the mean of the 5 points taken, and ranged between 20.6 and 25.4 cm. Values recorded were used to calculate GHG fluxes from each chamber after the insertion in the soil.

On each sampling occasion, the chamber was closed for 40 min. Ten ambient air samples were taken (5 at the start of the chamber closure, and 5 at the end of the 40 min period) to provide background values for N_2O, and stored in pre-evacuated 20 ml glass vials. The average of N_2O, CH_4 and CO_2 concentrations in these ambient air samples was used as the time zero sample (T0). After 40 min (T40) the chamber headspace was sampled via the three-way valve on the chamber lid. 60 ml gas samples were taken from each chamber headspace (and from ambient air) and transferred to 20 ml pre-evacuated glass vials, using a syringe and hypodermic needle to inject the sample into the vial. A second needle was used to release the sample to ambient pressure. Soil surface temperature inside chambers was also recorded every sampling date and the data used to calculate the gas fluxes.

We assumed a linear accumulation of headspace GHG concentrations in our flux calculations; an observation we have verified for N_2O using the same chambers on the same soil type following AN applications (Cardenas et al., 2010), thus using our resources to ensure we accounted for spatial variability of fluxes within each plot (using three chambers per plot), and opting to sample at high frequency (see next section), improving the ability to account for temporal variability of fluxes. In addition, data collected from a nearby experiment carried out at the same time as the current experiment, showed that CO_2 accumulation in the headspace was linear at 40 min in 88% of the chambers tested (L. Cardenas and D. Chadwick, personal communication).

2.5. Greenhouse gas flux measurements and laboratory analysis

Measurements of N_2O, CO_2 and CH_4 were conducted frequently over a 6-month period, from 16th May to 13th November 2011. Gas sampling was usually carried out between 10:00 and 12:00 h. Chambers were sampled every day during the first 15 days after AN and SL applications, and every two days until the next application. Gas samples were usually analyzed within 24 h of collection with a Perkin Elmer Auto-system gas chromatograph (GC500) equipped with two Elite Plot Q columns (30 m × 0.53 mm) and two detectors: ^{63}Ni electron capture detector (ECD) at 300 °C for measuring N_2O and a flame ionization detector (FID) at 350 °C for CO_2 equipped with a methanizer to analyze CH_4. This system was attached to an auto-sampler (Perkin Elmer headspace sampler Turbo matrix 110), which extracted a sample of 0.03 μl min^{-1} from the sampling vial and injected it into the GC. Calibrations were performed using standards of N_2O, CO_2 and CH_4 (0.33, 1.59, 5.23 ppm for N_2O; 2.97, 1198, 2467 ppm for CO_2; 2.05, 5.18, 10.18 ppm for CH_4). Concentrations of gases were calculated by comparing peak areas integrated with those obtained with the standards of each gas.

Fluxes were calculated from the accumulation of gas in the chamber as follows:

$$F = \rho \times H \times (C_{40} - C_0)/t \times 273.15/T \times 24 \times 10^4 \quad (1)$$

where F is the gas flux (kg N_2O-N ha^{-1} day^{-1}, g CH_4 ha^{-1} day^{-1}, kg CO_2-C ha^{-1} day^{-1}), ρ is the gas density (N_2O-N: 1.26 kg m^{-3}; CH_4: 717 g m^{-3}; CO_2-C: 0.536 kg m^{-3}) under STP conditions (273.15 K, 101,325 Pa), H is the effective height of the chamber (m), $C_{40} - C_0$ is the gas concentration at 40 min (T40) after chamber closure minus gas concentration of ambient sample (T0) (μm^3 m^{-3}), t is the time of chamber closure (40 min) and T is the temperature (K) inside the chamber. Cumulative emissions were calculated by the trapezoidal method (Cardenas et al., 2010; Van den Pol-van Dasselaar and Oenema, 1997; Velthof et al., 1996). For N_2O and CH_4, emissions from the zero N treatment were subtracted from the corresponding values in fertilized plots for the whole period of measurements, giving net emissions attributable to the N applied (for N_2O) or to the amount of slurry (for CH_4). Seasonal N_2O emission factors (EFs), expressed as % N applied to the soil, were calculated for each amendment studied during the experiment by dividing the net N_2O cumulative fluxes by the total N applied. Methane emission factors, expressed as % C applied in the soil, were calculated for each application of slurry by dividing the net cumulative CH_4 fluxes by the total amount of slurry applied.

2.6. Ammonia (NH_3) losses

Although NH_3 losses were not measured in the field, an estimation of the percentage of NH_3 loss from each application was calculated using the empirical model ALFAM (Ammonia Losses from Field-Applied Animal Manure) provided by Sogaard et al. (2002). The ALFAM model is a multiple regression model based on empirical data of NH_3 loss from experiments conducted in seven European countries (UK, Denmark, The Netherlands, Norway, Sweden, Switzerland and Italy) and it uses the Michaelis–Mentel type equation to predict NH_3 loss over time (t) after slurry application as:

$$N(t) = N_{max} \times (t/(t + K_m)) \quad (2)$$

where $N(t)$ is the cumulative loss fraction of total amoniacal nitrogen (TAN), N_{max} (as % NH_4^+-N applied) the total time integrated loss and K_m the time (h) in which 50% of NH_3 loss occurred. The instantaneous emission rate corresponds to the derivated dN/dt of Eq. (2) as follows:

$$dN/dt : N_{max} \times (K_m \times (t + K_m)^{-2} \quad (3)$$

For the estimation, variables related to slurry, (type of slurry (pig/cattle), application technique, DM and NH_4^+-N contents), weather (air temperature, wind speed) and soil conditions (dry/wet) are required model inputs because these significantly affect N_{max} and K_m.

In this experiment, NH_3 losses were estimated to provide an understanding of a major loss pathway of available N, which could be used to help explain the patterns of N_2O emissions observed. The value of N_{max} provided after each of the three slurry applications was considered as the cumulative NH_3 loss and expressed as percentage of NH_4^+-N applied with the slurry. Losses of NH_3 from AN applications were estimated using the emission factor of 1.4% (percentage of loss of NH_4^+-N applied) reported by Misselbrook et al. (2004), in grasslands fertilized with NH_4NO_3 and without grazing management.

2.7. Soil sampling and analysis

During the experiment, flux measurements were accompanied by measurements of soil moisture at a depth of 0–10 cm. Every

sampling day, one soil core per replicate of each treatment was taken and then unified into one sample per treatment for analysis of gravimetric soil moisture. This parameter was measured by oven-drying the samples at 105 °C for 24 h. Soil BD of the 0–10 cm layer was determined at the start of the experiment from five undisturbed blocks of soil with a mean height of 8.6 cm. Measures of the width and length of each side of block were recorded. The volume of soil was calculated and the soil was dried at 105 °C to constant weight to determine the dry weight. Bulk density was then calculated from the weight of the dry soil and the volume it occupied and expressed as Mg m^{-3}. Particle density (PD) was assumed to be 2.65 Mg m^{-3}. Porosity was calculated according to Eq. (4):

$$\text{Porosity} = 1 - (\text{BD}/\text{PD}) \quad (4)$$

Gravimetric soil water content was then converted to % soil water filled pore space (WFPS) using the following equation (5):

$$\text{WFPS} = ((\text{Water content} \times \text{BD})/\text{Porosity}) \times 100 \quad (5)$$

Soil ammonium (NH_4^+-N) and nitrate (NO_3^--N) contents were analyzed once a week. Five cores (0–10 cm) per plot were taken from the soil sampling zone in each plot, extracted with 2 M KCl (Searle, 1984) for 1 h at 200 rpm in a horizontal shaker (1:2 fresh soil:extractant ratio) and then filtered through Whatman No. 5 paper. Extracts were analyzed for NO_3^--N and NH_4^+-N soil contents using a SKALAR SAN^{Plus} analyser (provided with a SKALAR 5000-02 analyser unit, a SKALAR 28503902_0 photometric detector, a SKALAR 1050d auto-sampler and a circulating water bath).

2.8. Slurry analysis

Slurry samples were analyzed for total N by Kjeldahl (AOAC, 1990). Slurry density was calculated on the basis of the volume occupied by a mass of sample. The pH of the slurry was measured directly with a pH meter (HANNA HI 9025). Total C was also analyzed using a TOC analyzer (Skalar FormacsHT, Breda, The Netherlands). Dry matter (DM) was determined after 24 h of drying at 105 °C. Organic matter (OM) was estimated by loss on ignition (LOI); oven-dried samples (105 °C) were weighed and then ashed for 24 h at 550 °C in a muffle furnace. LOI was calculated as follows:

$$\text{LOI}\,(\%) = ((\text{oven-dried sample weight} - \text{ashed sample weight})/\text{oven-dried sample weight}) \times 100 \quad (6)$$

Mineral nitrogen (NO_3^--N and NH_4^+-N) was extracted using 2 M KCl. Slurry extracts were analyzed using a SKALAR SAN^{Plus} analyser (described in soil sampling and analysis section). Analysis of other macronutrients (P, K, Ca, Mg, S) were carried out by a commercial laboratory using the aqua-regia method (ISO, 1995) followed by determination by inductively coupled plasma optical emissions spectrometry (ICP-OES). Chemical and physical properties of the cattle slurry applied to the soil are summarized in Table 1.

2.9. Grass yield and composition

All plots were harvested three times, 21st June, 24th August and 31st October, using a reciprocating mower to cut an area of 2 × 1 m^2, at a cutting height of 5 cm. Fresh grass samples were taken from the harvesting zone for analysis for DM. This parameter was determined after 16 h drying at a temperature of 80 °C. Dried and ground grass samples were also analyzed for total N. Both parameters were used to determine N plant offtake after each cut.

2.10. Meteorological data

Total daily rainfall, daily average air and soil (10 cm depth) temperatures were recorded between 22nd June and 13th November by a weather station within 500 m of the experimental area. Also, the average wind speed (at 10 m height) for the day of the slurry addition and the following day was used as a model input to estimate NH_3 losses by the ALFAM model, after each application. Average monthly rainfall, air and soil temperature data for the last 40 years (1961–2000) were used to compare the weather conditions observed during the experiment.

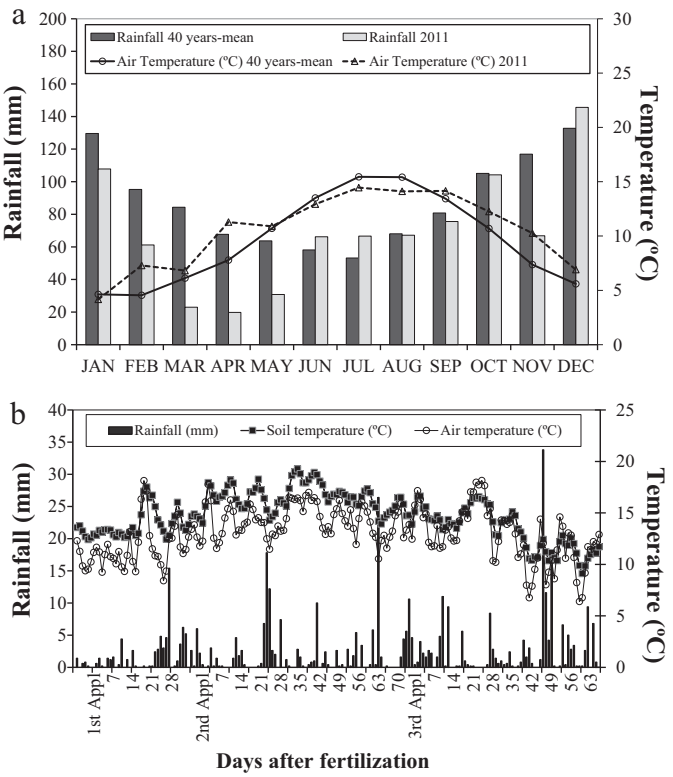

Fig. 1. (a) Mean monthly rainfall and air temperature in 2011 and the 40-year averages (1961–2000). (b) Total daily rainfall and average daily air and soil temperatures recorded during the experiment.

2.11. Statistical analysis

Statistical analyses were performed using Sigmaplot (11.0). One way ANOVA was used to study the effect of fertilization with NH_4NO_3 or cattle slurry on cumulative GHG emissions following each fertilization event, and for the total experiment. Two way repeated measures ANOVA was used to compare the same effect on mean N_2O, CH_4 and CO_2 fluxes under the different weather conditions and to determine significance of changes in soil (mineral N and WFPS) and grass yields (DM, % N contents, N offtake) during the experiment. Daily fluxes of N_2O, CH_4 and CO_2 were related to corresponding soil and weather parameters using Pearson correlation analysis. The "*r*" value provided by Sigmaplot was used to prove the influence of these environmental conditions on the pattern of GHGs.

3. Results

3.1. Weather conditions

The annual mean air temperature in 2011 was on average 0.9 °C higher than the 40-year mean value (Fig. 1a), Compared to the 40-year average (1961–2000),

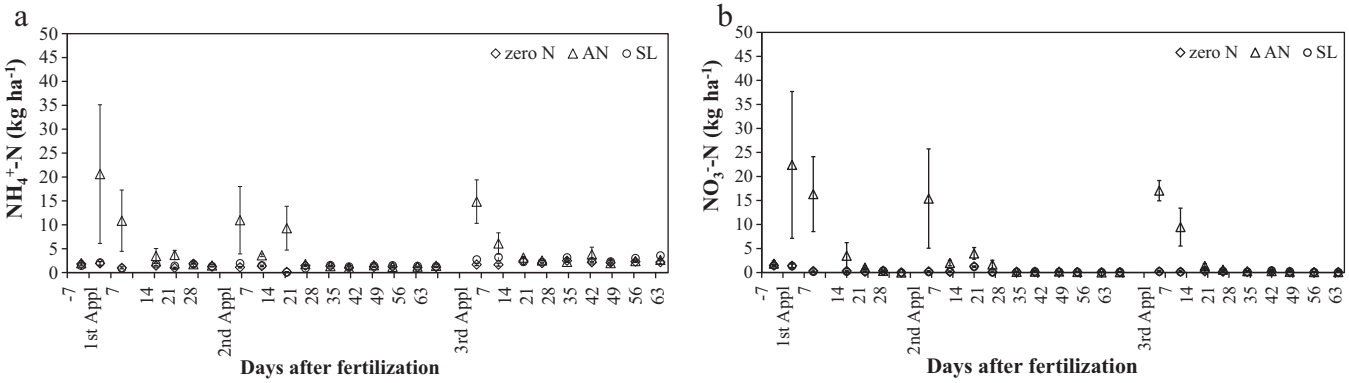

Fig. 2. Soil inorganic NH_4^+-N (a) and NO_3^--N (b) contents in the fertilized and control treatments during the 6-month study. Treatments: (zero N) unfertilized or control; (AN) ammonium nitrate; (SL): slurry. Each point represents the mean value of three replicates ± standard error.

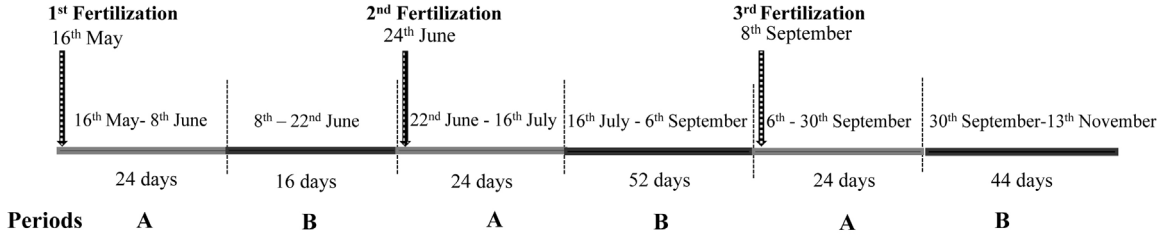

Fig. 3. A schematic representation of the time periods studied between N applications. The two groups of periods are represented by the letters 'A' and 'B'. Arrows represent the time of fertilization.

2011 was characterized by warm temperatures in spring (March–May) and autumn (September–November), and relatively cool conditions during summer (June–August). The mean daily air temperature during the sampling period (from 16th May to 13th November 2011) ranged from 6.4 °C (6th November) to 18.2 °C (2nd October) (Fig. 1b). November was the coldest month of the experimental period with a mean monthly temperature of 10.3 °C, whereas maximum mean monthly temperature was recorded in July (14.5 °C).

Soil temperature ranged from 9.1 °C (7th November) to 19.3 °C (28th July). Daily soil temperature was around 14 °C in the period after the first N-fertilization and increased to mean values of 16.5 °C in the period after the second application, coinciding with the summer growing season. Mean soil temperature decreased to 13.3 °C, after the third N application, which coincided with the arrival of the autumn.

Rainfall in 2011 was low compared with the 40-year mean (21% less rainfall) (see Fig. 1a), particularly in the months of March, April and May (before the first N application) when the precipitation was only 73%, 71% and 52% of the 40-year average. Total precipitation during the sampling period was 427 mm, (76, 166 and 186 mm fell during the period after the first, second and third N-fertilization event, respectively) with October as the wettest month and May as the driest. Two substantial precipitation events (more than 20 mm day^{-1}) were recorded on 28th August and on 24th October (Fig. 1b).

3.2. Soil mineral N

After each of the three fertilizer applications in the experiment, only high soil NH_4^+-N values were found in the AN plots (Fig. 2a), with maximum values of 20.6, 11.0 and 14.8 kg N ha^{-1} in the first, second and third application, respectively. Values of between 0.2 and 2.5 kg N ha^{-1} were found in the zero N plots and between 0.1 and 3.6 kg N ha^{-1} in the SL plots. Also, the highest soil NO_3^--N contents were found in AN plots (Fig. 2b), with maximum values of 22.4, 15.4 and 17.0 kg N ha^{-1} after the first, second and third N-fertilization, respectively. Zero or very low values of NO_3^--N were recorded in the zero N and SL plots, with values in the range of 0.0–1.4 kg N ha^{-1} in zero N plots and 0.0–1.3 kg N ha^{-1} in SL plots. Two way repeated measures ANOVA showed statistical differences between treatments in NH_4^+-N and NO_3^--N contents with date of sampling ($P < 0.001$) and these contents were significantly higher in AN plots within the first three weeks after each application event. No statistical differences were found between treatments in the soil mineral N contents after this time (for each N-fertilization) ($P > 0.05$).

Based on the significant statistical increases in soil NH_4^+-N and NO_3^--N concentrations during the first three weeks after each fertilization event, GHG emissions data recorded in this experiment were collated into two periods within each fertilization event (Fig. 3). The period corresponding to the first three weeks of measurements (24 days) after each N application was designated as period 'A', and the remaining days until the next fertilization or end of the experiment, designated as period 'B'.

3.3. Soil WFPS

Low %WFPS values were recorded in the period after the first two fertilization dates (Fig. 4a). The dry conditions during the spring and summer resulted in WFPS values ranging from 35% to 65% and from 37% to 67% after the first and second application, respectively. These values were below the threshold range of 60–70% WFPS, where denitrification becomes dominant and high N_2O fluxes are often observed (Bateman and Baggs, 2005; Davidson, 1993; Dobbie et al., 1999; Linn and Doran, 1984). After the third application, there was an increased frequency of rainfall events so %WFPS increased to values ranging from 54% to 93% WFPS, which were significantly greater than the WFPS following the first two fertilization occasions ($P < 0.001$). Statistical analysis showed that the mean soil WFPS value during the latter phase after the autumn application (period 'B') was the highest of the experiment ($P < 0.001$) (Fig. 5a). However, there were no significant differences in the values of %WFPS of soil samples from zero N, AN and SL plots within each sampling date ($P > 0.05$) and within each period ($P > 0.05$).

3.4. Greenhouse gas fluxes

A total of 112 GHG flux samplings were carried out over the 6-month period (16th May–13th November 2011); 25 after the first N-fertilizer application and 43 and 44 after the second and third applications, respectively. Fluxes of N_2O were low throughout the whole experiment in all treatments studied (Fig. 4b). Zero N plots resulted in values from −0.004 to 0.006 kg N_2O-N ha^{-1} day^{-1}. Fluxes from AN and SL plots ranged from −0.005 to 0.008 kg N_2O-N ha^{-1} day^{-1} and from −0.004 to 0.004 kg N_2O-N ha^{-1} day^{-1}, respectively. The applications of AN and SL were not followed by a distinct peak of emission following the first application in May. The highest values were observed 15 days after the application of AN and SL and after a period of some rainfall events <5 mm. After the second application, increases in N_2O fluxes were observed on the fourth (higher in AN plots) and twenty fourth days (after amendment applications). After the third application, only AN plots showed an increase in N_2O fluxes from the 10th day to the 14th day, during which time maximum N_2O fluxes were recorded. Forty-six days after the third application of the treatments small peaks of N_2O in zero N and SL plots were observed. This continued for 2 further days from the AN plots only.

Fluxes of CH_4 (Fig. 4c) were generally negative or close to zero from the zero N plots and those fertilized with AN. Values ranged between −11.5 and 6.4 g CH_4 ha^{-1} day^{-1} and between −10.8 and 4.1 g CH_4 ha^{-1} day^{-1} from the zero N and AN treatments, respectively. Only plots fertilized with SL showed distinct peaks of CH_4 fluxes between days 1 and 3 following each slurry application. Values ranged from −9.8 to 304.5 g CH_4 ha^{-1} day^{-1}. Maximum CH_4 fluxes appeared immediately after each SL application, with values of 65.4, 129.6 and 304.5 g CH_4 ha^{-1} day^{-1} in May, June and September, respectively.

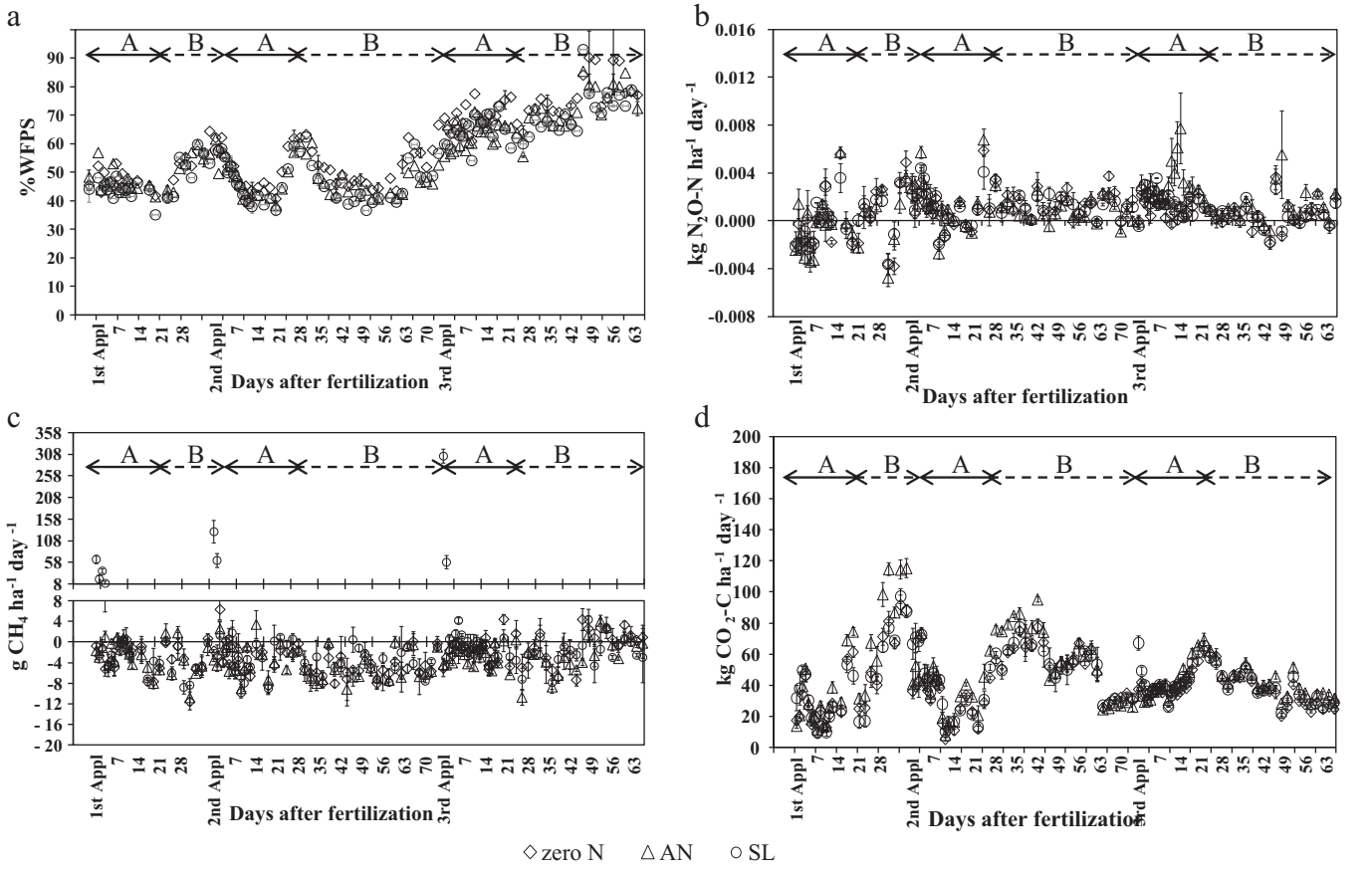

Fig. 4. (a) % Water filled pore space (WFPS) and daily fluxes of N_2O (b), CH_4 (c) and CO_2 (d). Letters 'A' and 'B' represent the different periods after fertilization and horizontal arrows, their length. Periods – 1st fertilization: 'A' (16th May–8th June) and 'B' (8th–22nd June); 2nd fertilization: 'A' (22nd June–16th July) and 'B' (16th July–6th September); 3rd fertilization: 'A' (6th–30th September) and 'B' (30th September–13th November). Treatments: (zero N) control; (AN) ammonium nitrate; (SL) slurry. Each point represents the mean value of three replicates ± standard error.

Fluxes of CO_2 (Fig. 4d) ranged from 5.7 to 91.8, from 8.3 to 115.6 and from 9.9 to 97.4 kg CO_2-C ha^{-1} day^{-1} from the zero N, AN and SL treatments, respectively. Immediately after grass was harvested, CO_2 fluxes were low. Maximum CO_2 peaks in the whole experiment were observed after the first N application.

3.5. Cumulative GHG fluxes

Total cumulative fluxes of N_2O, CO_2 and CH_4 for the whole experimental period (16th May–13th November) and in the periods between each fertilizer application and the next fertilization or end of the experiment (1st fertilization: 'A' (16th May–8th June) + 'B' (8th June–22nd June); 2nd fertilization: 'A' (22nd June–16th July) + 'B' (16th July–6th September); 3rd fertilization: 'A' (6th September–30th September) + 'B' (30th September–13th November)) are shown in Table 2. Also, mean cumulative N_2O, CH_4, and CO_2 fluxes (Fig. 5b, c and d, respectively) were explored statistically for the periods 'A' and 'B' after each fertilization (see previous definition of these periods).

Total N_2O cumulative fluxes from the AN plots resulted in slightly higher fluxes than those recorded from the zero N and SL treatments, but these differences were not statistically different ($P > 0.05$). Plots fertilized with AN showed significant differences in mean cumulative N_2O fluxes ($P < 0.05$) in the period 'A' after the 3rd N-fertilization compared with those fluxes from the zero N and SL plots (Fig. 5b). Mean N_2O fluxes from AN plots were 44% and 53% higher than those from SL and zero N, respectively. In terms of N_2O EFs, the total application of 240 kg N ha^{-1} with AN and SL caused a total N_2O-N loss during 6-months measurements of 0.21 and 0.17 kg N ha^{-1}, respectively, accounting for 0.02% and 0.003% of the N applied.

Statistically significant differences were found in total CH_4 cumulative fluxes (Table 2) between treatments ($P < 0.001$). Plots fertilized with slurry resulted in fluxes of 0.41 kg CH_4 ha^{-1}, with negative fluxes from the other treatments (−0.52 and −0.60 kg CH_4 ha^{-1}, from zero N and AN plots, respectively). Mean cumulative CH_4 emissions from SL were significantly greater ($P < 0.001$) compared with zero N and AN treatments in the periods 'A' (Fig. 5c). The quantity of CH_4 emitted in period 'A' increased with successive SL applications, with the third application resulting in the greatest flux. In the periods 'B', no differences were found ($P > 0.05$) between treatments. Indeed during these periods ('B'), the SL plots resulted in negative cumulative CH_4 fluxes, similar to those from the zero N and AN plots. Comparing the mean CH_4 cumulative fluxes from periods 'B' after fertilization, we found that CH_4 uptake by the soil was significantly lower after the third application compared with the first and second fertilization ($P < 0.001$). The application of cattle slurry resulted in CH_4 emission factors representing 0.01, 0.04% and 0.06% of the C applied following each application, and a total for the whole experiment of 0.04% C applied (kg CH_4-C/kg slurry-C).

Table 2
Cumulative fluxes after the three fertilizations and total cumulative fluxes of N_2O (kg N_2O-N ha^{-1}), CH_4 (kg CH_4 ha^{-1}) and CO_2 (Mg CO_2-C ha^{-1}). Mean value of three replicates and standard error (between brackets). Treatments: (zero N) control; (AN) ammonium nitrate; (SL) slurry. Fertilizations: 1st: 'A' (16th May–8th June) + 'B' (8th June–22nd June); 2nd: 'A' (22nd June–16th July) + 'B' (16th July–6th September); 3rd: 'A' (6th September–30th September) + 'B' (30th September–13th November); total (16th May–13th November). Treatments with different letter within the same row are statistically different ($P < 0.05$).

		Treatment		
Gas	Fertilizations	Zero N	AN	SL
N_2O	1st	0.007 (0.009) a	0.006 (0.003) a	0.007 (0.007) a
	2nd	0.105 (0.026) a	0.089 (0.008) a	0.102 (0.020) a
	3rd	0.048 (0.010) b	0.115 (0.022) a	0.058 (0.006) b
	Total	0.160 (0.045) a	0.205 (0.031) a	0.166 (0.019) a
CH_4	1st	−0.13 (0.01) a	−0.12 (0.01) a	−0.02 (0.02) a
	2nd	−0.31 (0.03) b	−0.32 (0.02) b	0.01 (0.08) a
	3rd	−0.08 (0.02) b	−0.17 (0.04) b	0.42 (0.01) a
	Total	−0.52 (0.02) b	−0.60 (0.03) b	0.41 (0.10) a
CO_2	1st	1.59 (0.09) a	2.04 (0.02) a	1.56 (0.14) a
	2nd	3.42 (0.09) a	3.91 (0.16) a	3.60 (0.34) a
	3rd	2.61 (0.04) a	2.90 (0.16) a	2.79 (0.12) a
	Total	7.61 (0.02) a	8.85 (0.22) a	7.96 (0.59) a

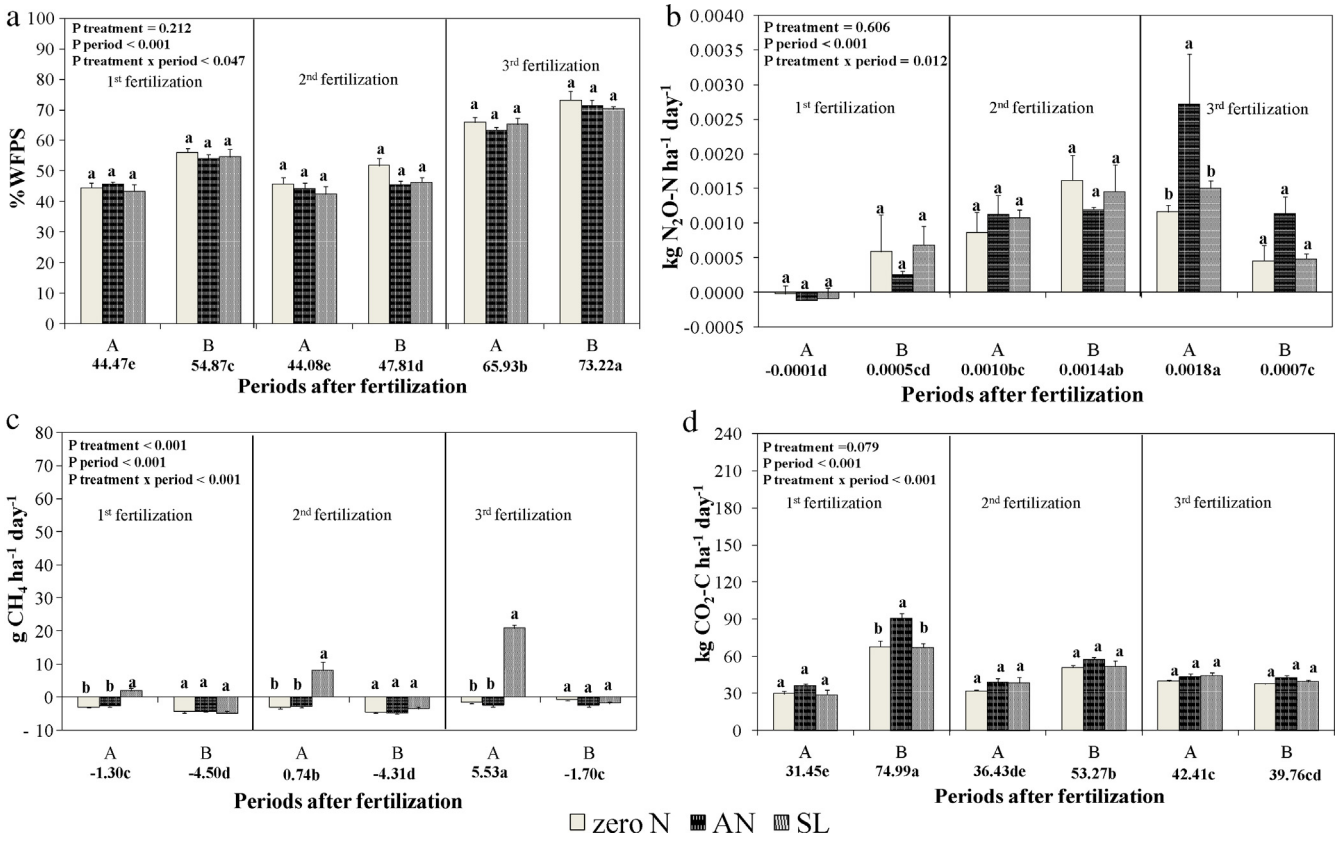

Fig. 5. Results of a two way ANOVA repeated measures and pairwise multiple comparison test (Tukey test) for mean cumulative fluxes of (a) N_2O, (b) CH_4 and (c) CO_2. Bars and error bars show the mean and standard error ($n = 3$). Values below the *x*-axis show mean value of the periods ('A', 'B'). Means and bars with the same letter show no significant difference using Tukey test ($P > 0.05$). Treatments: (zero N) control; (AN) ammonium nitrate; (SL) slurry. Periods – 1st fertilization: 'A' (16th May–8th June) and 'B' (8th–22nd June); 2nd fertilization: 'A' (22nd June–16th July) and 'B' (16th July–6th September); 3rd fertilization: 'A' (6th–30th September) and 'B' (30th September–13th November).

Total cumulative CO_2 fluxes (Table 2) from the amendments were not statistically different to those from the zero N plots ($P > 0.05$). The AN treatment resulted in significantly different mean CO_2 fluxes compared with the other treatments (Fig. 5d) in the period 'B' after the first application (6th-22nd June) ($P < 0.05$), with mean cumulative values of 80.8 kg CO_2-C ha^{-1} day^{-1}, 20% and 22% higher than zero N and SL, respectively.

3.6. Correlations between GHG fluxes and soil and weather parameters

The daily N_2O fluxes observed in all treatments showed significant positive correlation with daily %WFPS ($r = 0.17$; $P < 0.01$), soil ($r = 0.06$; $P < 0.05$) and air temperature ($r = 0.08$; $P < 0.05$). Also, negative correlations were observed between the soil mineral N values and the corresponding N_2O fluxes observed from the treatments at each soil sampling day (NH_4^+-N: $r = -0.17$; $P < 0.01$ and NO_3^--N: $r = -0.13$, $P < 0.05$).

Daily CH_4 fluxes from zero N ($r = 0.39$; $P < 0.01$) and AN plots ($r = 0.21$; $P < 0.01$) were related to daily WFPS. Soil temperature was negatively correlated with the daily CH_4 fluxes from zero N and AN plots after the first ($r = -0.25$; $P < 0.01$) and second fertilization ($r = -0.22$; $P < 0.01$). Also, soil N-NH_4^+ contents were related to the corresponding CH_4 fluxes at the day of the soil sampling from zero N ($r = 0.40$; $P < 0.01$) and AN plots ($r = 0.23$; $P < 0.01$).

Daily CO_2 fluxes from all treatments showed significant correlations with daily soil temperatures after each fertilization (r values in each fertilization of 0.43, 0.34, 0.57; $P < 0.01$) and also with soil WFPS (r values in each fertilization of 0.43, 0.34, 0.57; $P < 0.01$).

3.7. Losses of NH_3

On the basis of the output from the ammonia emission model (ALFAM), the slurry application under the warm and dry weather conditions of the experiment may have led to significant volatilization of NH_3. As some studies report (e.g., Pfluke et al., 2011; Thompson et al., 1990) volatilization tends to occur within the first 24 h after application to soil. Wind speed values used as the model input data were 5 m s^{-1} for the first application and 4 m s^{-1} in the other two fertilization events. With respect to air temperature, the surface broadcasting of slurry was carried out when mean air temperatures were 11, 14, 17 °C, at the time of the first, second and third slurry applications. Values for DM and the NH_4^+-N content of the slurry applied in each fertilization (Table 1) were input into the ALFAM model. According to the model, the interaction of these input parameters may have caused losses of NH_3 of 86%, 69% and 82% (% of NH_4^+-N applied) after each slurry application, thus leaving little NH_4^+-N in the soil at risk of loss as N_2O or for plant offtake. In the AN plots, the application of 80 kg N ha^{-1} of NH_4NO_3 (40 kg ha^{-1} as NH_4^+-N) may have resulted in a loss of 0.6 kg N ha^{-1} via NH_3 volatilization after each fertilization.

3.8. Dry matter yield and N plant offtake

Dry matter yields were statistically greater ($P < 0.001$) for the first two herbage cuts (22nd June and 24th August) than the third cut, for all treatments (Fig. 6a). The application of AN and SL significantly increased DM in the first and second harvests ($P < 0.001$) compared with the zero N plots. Dry matter yields in SL were 56% greater than yields from the zero N plots in August ($P < 0.001$). No differences between treatments ($P < 0.001$) were found for the DM yields after the third harvest (31st October). For each cut, the herbage N contents were similar between the zero N, AN and SL treatments. Highest total N herbage concentrations (mean value of 3.59% DM) were found in grass from the October harvest (Fig. 6b), compared with the June and August harvests ($P < 0.001$). So, N offtake (Fig. 6c) in the first two applications were statistically greater ($P < 0.05$) compared with the third application, for all treatments. Similar to DM yield, the AN plots significantly increased N offtakes in the first and second cut ($P < 0.05$) compared with the zero N and SL plots. The SL treatment resulted in significantly greater N offtake (58% more) compared with N offtake on the zero N plots after the second harvest ($P < 0.05$). At the October grass harvest, no differences in N offtake were found between N fertilization treatments ($P > 0.05$).

3.9. Nitrogen budget

Nitrogen inputs and outputs, measured or estimated (NH_3 volatilization), for each period after fertilization and also for the whole experiment are summarized in Table 3. The initial soil mineral N content for each period was taken into account to provide an indication of the available mineral N pool for plant uptake. The soil sample taken on 13th May prior to the first fertilization was considered as the initial soil N content for the first fertilization and for the entire experiment. For the second

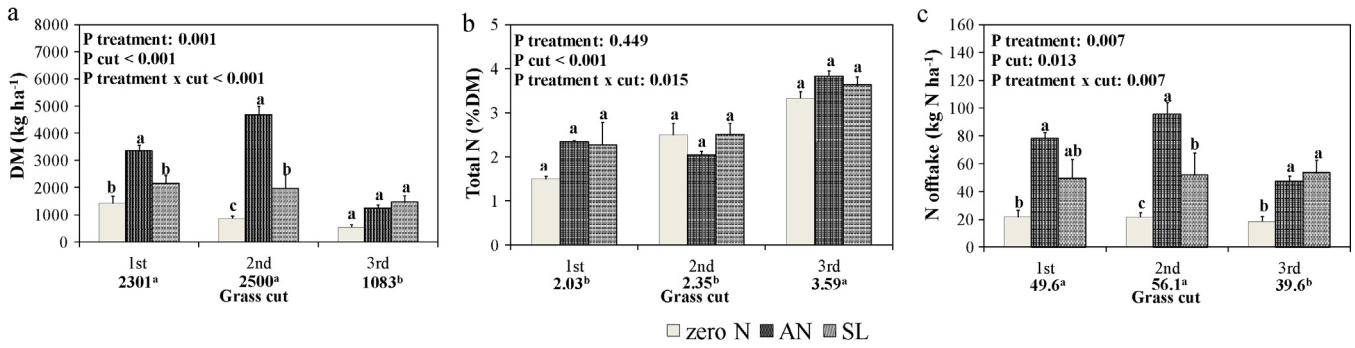

Fig. 6. Results of a two way ANOVA repeated measures and pairwise multiple comparison test (Tukey test) for grass (a) dry matter (DM) yield expressed as kg ha^{-1}, (b) total plant N concentration expressed as %DM, and (c) N offtake (kg N ha^{-1}) in the three cuts carried out in the experiment. Values below the *x*-axis show the mean value of the treatments (zero N, AN, SL) within each grass cut (1st, 2nd, 3rd). Means and bars with the same letter show no significant difference using Tukey test ($P > 0.05$). Treatments: (zero N) control; (AN) ammonium nitrate; (SL) slurry. Grass harvests: 1st (21st June), 2nd (24th August), 3rd (31st October).

Table 3
Initial soil N contents, N inputs and outputs (measured or estimated) and the N balance in the periods studied after each fertilization, and for the whole experiment. Units expressed as kg N ha^{-1}. Treatments: (zero N) control; (AN) ammonium nitrate; (SL) slurry. 1st fertilization: (16th May–22nd June); 2nd fertilization: (22nd June–6th September); 3rd fertilization: (6th–13th November); total experiment (16th May–13th November).

	1st fertilization			2nd fertilization			3rd fertilization			Total experiment		
	Zero N	AN	SL	Zero N	AN	SL	Zero N	AN	SL	Zero N	AN	SL
NH_4^+-N	1.8	2.0	1.5	1.2	1.5	1.3	1.2	1.4	1.3	1.8	2.0	1.5
NO_3^--N	1.5	1.9	1.5	0.0	0.0	0.0	0.0	0.1	0.2	1.5	1.9	1.5
Total initial soil mineral N content	3.3	3.9	3.0	1.2	1.5	1.3	1.2	1.5	1.5	3.3	3.9	3.0
Inputs												
NH_4^+-N	0.0	40.0	35.1	0.0	40.0	28.4	0.0	40.0	28.9	0.0	120.0	92.4
NO_3^--N	0.0	40.0	0.4	0.0	40.0	0.0	0.0	40.0	0.0	0.0	120.0	0.4
Organic-N	0.0	0.0	44.5	0.0	0.0	51.5	0.0	0.0	51.1	0.0	0.0	147.1
Total N inputs	0.0	80.0	80.1	0.0	80.0	79.8	0.0	80.0	80.1	0.0	240.0	240.0
Outputs												
N_2O-N	0.007	0.006	0.007	0.105	0.089	0.102	0.048	0.115	0.058	0.160	0.205	0.166
NH_3-N volatilization[a]	0.0	0.6	30.1	0.0	0.6	19.7	0.0	0.6	23.6	0.0	1.8	73.4
N plant offtake	21.7	77.9	49.3	21.6	95.2	51.5	18.3	47.3	53.1	83.3	220.4	153.9
Total N outputs	21.7	78.5	79.4	21.7	95.8	71.3	18.3	48.0	76.8	61.7	222.3	227.5
N balance	−18.4	5.4	3.7	−20.5	−14.3	9.8	−17.1	33.5	4.8	−58.4	21.6	15.5

[a] Estimated using the ALFAM model (Sogaard et al., 2002).

and third fertilization events, the soil mineral N values were from the last soil sample taken prior to the next fertilization.

Zero N plots showed a negative N balance after each of the three fertilization periods. The AN treatment resulted in a N deficit of 14.3 kg N ha^{-1} in the period studied after the second fertilization. In the first and third periods, surpluses of N were observed, with the highest surplus (33.5 kg N ha^{-1}) occurring after the third application. For the SL treatment, surpluses ranged from 3.7 to 9.8 kg N ha^{-1}. Across the entire measurement period (taking all three periods into account), only the zero N plots resulted in N deficit (negative values), unlike the AN and SL plots where soil remained with N surpluses of 21.6 and 15.5 kg N ha^{-1}, respectively.

4. Discussion

4.1. Fluxes of N_2O

The low N_2O emissions observed in our experiment in 2011 were most likely the consequence of low %WFPS, high soil and air temperatures and low available soil N contents. As already mentioned, 2011 was considered a very dry year compared with the 40-year mean for rainfall (Fig. 1a), especially before and immediately after the first fertilizer application. The low rainfall after the first fertilization (20% less than the 40-year mean for May and June in the SW England) meant that the WFPS did not increase above 60%, resulting in frequent negative N_2O fluxes (as low as −0.005 kg N_2O-N ha^{-1} day^{-1}). Rain fell just before the second and third fertilizer applications, resulting in an immediate increase of the WFPS above 60% (the threshold value above which anaerobic activity becomes significant, Linn and Doran, 1984), and subsequent increases in N_2O fluxes (Davidson, 1993; Dobbie et al., 1999). Between the second and third applications, the WFPS varied between 35% and 60% producing low N_2O fluxes, probably as result of nitrification (Bateman and Baggs, 2005). The low N_2O emissions during period 'B' after the third fertilization were due to high %WFPS values (near saturation), which would have promoted complete denitrification to N_2 (Rudaz et al., 1999). In period 'A' after the third fertilization, where soil %WFPS was optimal for N_2O production (mean of 66% WFPS), there was only a small increase of up to 0.008 kg N_2O-N ha^{-1} day^{-1} from the AN treatment. But there was no increase in N_2O flux from the SL treatment, probably because of the lower NO_3^--N content in the SL treatment and/or possibly because the addition of available C in the SL treatment enhanced full denitrification to N_2 (Weier et al., 1993).

The low N_2O fluxes observed during the 2011 experiment contrasted with previous experiments carried out in the South West of England. The North Wyke meteorological records show that during the past 15 years (1995–2010), 2003 was a similar dry year, especially in the spring and summer. Smith et al. (2012) reported low N_2O emissions (0.15–0.22 kg N ha^{-1}) from cut grassland from an experiment on the same soil type in the same region in 2003, following applications of 80–100 kg N ha^{-1} as AN in spring and summer. They attributed these low N_2O emissions to the low %WFPS. However, their emissions in 2003 were still 25 and 2.5-fold greater than those measured in our experiment in 2011

after the applications in May and June, respectively. Smith et al. (2012) also reported an increase in N_2O emissions during the same months following NH_4NO_3 applications in 2004, which was considered a wet year, more typical of SW England. The amount of N_2O emitted in 2004 respect to 2003 was approximately 9 and 4-fold higher in the applications of May and June, respectively. Cardenas et al. (2010), also, reported frequent and high N_2O fluxes in 2006 (up to 200 g N_2O-N ha^{-1} day^{-1} in June) after the application of 75 kg N ha^{-1} as NH_4NO_3 in a grazed grassland on the same soil type, close to our experimental site. They ascribed these high N_2O fluxes to intense denitrification due to the anaerobic soil conditions generated by the large amount of rainfall recorded. Chadwick et al. (2000) observed large differences in N_2O emissions following slurry application under different weather conditions at a site close to ours. They also observed that the amount of N_2O emitted was 10 times lower in July, under warmer conditions (WFPS values below 60%), than in April, probably due to the relatively dry (aerobic) nature of the soil.

Unlike our experiment, the N_2O fluxes from the experiments cited above (on the same soil and conducted at the same time as our experiment) were not limited by soil mineral N, only by the WFPS. In this sense, the loss of NH_4^+-N through plant offtake and NH_3 volatilization contributed to the low soil NH_4^+-N observed during our experiment and, therefore, low level of soil NO_3^--N. In our study, N_2O emissions under the limited conditions of anaerobicity and soil NO_3^--N content resulted in emission factors lower than the IPCC default value of 1% (IPCC, 2006), and 8- and 40-fold lower than the lowest emission factors reported in the experiments carried out in North Wyke and cited above for NH_4NO_3 and cattle slurry, respectively.

4.2. Other N losses

Converting the emissions to proportion of total N, the ALFAM model estimated that 25–38% of the total N in the slurry was lost by NH_3 volatilization. These results contrasted with the estimated 1% following NH_4NO_3 application (Misselbrook et al., 2004). The climatic conditions (warm and windy), and the application method (surface broadcasting) coupled with the high DM content of the slurry would have enhanced NH_3 volatilization from SL plots. Surface broadcasting of slurry results in greater NH_3 losses compared to other application methods such as band spreading, use of trailing hose or shallow injection (e.g., Huijsmans et al., 2003; Misselbrook et al., 1996; Pfluke et al., 2011; Smith et al., 2000) due to the increased surface area of the slurry in contact with the air. Also, the absence of rainfall immediately after the slurry spreading reduced the infiltration of the slurry ammonium into the soil (Misselbrook et al., 2005). For the same reason, the high slurry DM content could have an important effect on NH_3 loss (Misselbrook et al., 2005; Pain et al., 1989); the DM content of the slurry used in our experiment was at the value suggested by Sommer and Olesen (1991), which promotes NH_3 emissions. The application under windy conditions would have enhanced the mass transfer and gas exchange between the slurry and the atmosphere (Misselbrook et al., 2005).

The warm and dry climatic conditions during the experiment stimulated plant growth on all treatments and thus generated a high plant N demand. For the zero N plots, the soil N deficits observed meant that plant N requirement was probably met via the mineralization of soil OM. For the SL fertilized plots, plant offtake represented 64% (mean value) of the total N applied in each fertilization. Moreover, plant N offtake seems to have been influenced by losses of N via NH_3 volatilization on the SL treatment, where the lowest plant offtake observed (after the first application) coincided with the highest NH_3 volatilization predicted, resulting in a much reduced soil NH_4^+-N content available for plant requirements. Misselbrook et al. (1996) reported data about NH_3 volatilization and N offtake from an experiment carried out at a sward located in SW England after surface application of cattle slurry. Their data showed that the lowest N offtake (23.4 kg N ha^{-1}) was in June coinciding with the highest NH_3 losses (30.6 kg N ha^{-1}). Also, Carran et al. (1982) observed that the soil conditions directly influence the proportion of N lost as NH_3 and N offtake by the plant. In the AN treatment, most of the N applied was taken up by the grass, especially after the first two harvests, when the plant N requirements are highest. In addition, after the second application the grass required more N than the N applied, suggesting mineralization of the OM supplied some the N required by the plant. The decrease in N offtake observed in the final harvest can be explained by the increase in soil %WFPS after the third fertilization. Losses of N could have limited the amount of N available for the plant. This effect was observed by Abassi et al. (2005) in a permanent grassland in Wales on soil in saturated conditions.

The N budget in the AN treatment showed a surplus (33.5 kg N ha^{-1}) at the end of the experiment. From this surplus, only 2.8 kg N ha^{-1} remained in the soil as mineral N. We assumed negligible losses of N via nitrate leaching during our measurement period, as there was little opportunity for leaching to occur. So, the surplus observed in AN could be attributed to losses as N_2 due to the anaerobic conditions caused by the high %WFPS after the third application. The resulting fraction of N applied attributed to N_2 (42% of the N applied) is higher than the range observed (8–25.5%) by Estavillo et al. (1994), Rudaz et al. (1999) and Velthof et al. (1996) after application of mineral fertilizers in grasslands. Compared to AN, the SL treatment did not result the same surplus due to the high plant N offtake and the estimated losses of N by NH_3 volatilization.

4.3. Fluxes of CH_4

Methane emissions were observed immediately after the application of slurry. Fluxes were short-lived, occurring within the first two (fertilization of June and September) to three days (in May) after application of the material. The larger mean CH_4 cumulative fluxes in the later applications could be related to the different DM content of the slurries applied, and the environmental conditions. The DM content of the slurry applied in September (6.5%) was lower than the DM of the slurry applied in May. Lower DM contents could enhance slurry infiltration in the soil promoting methanogenesis due to greater anaerobicity. This was not the case for the June application compared to September because DM contents were similar. We suggest that soil moisture was the controlling factor in the CH_4 production due to lower %WFPS observed in the June application.

In the periods between application (periods 'B') the soil acted as a net sink, resulting in negative fluxes as low as -9.8 g CH_4 ha^{-1} day^{-1}. Similar conclusions concerning the CH_4 fluxes from the soil after cattle slurry applications were reported by Chadwick et al. (2000), Jones et al. (2005) and Rodhe et al. (2006). Emissions of CH_4 from the AN plots were not observed, with negative fluxes often measured, indicating consumption of atmospheric CH_4. Soil moisture had an influence in gas diffusivity in the soil and, therefore, controlled the amount of CH_4 available for the oxidation by metanotrophic bacteria (Dunfield, 2007). In the periods following the first and second application, methanogenesis would have been suppressed in favor of methanotrophy due to the aerobic conditions generated by the low %WFPS in the zero N and AN plots. Pearson correlations between WFPS and CH_4 consumption rates observed in these treatments seem to confirm this assumption. Methane uptake rates generally increase with decreasing soil moisture because molecular diffusion in water is a factor of 10^4 times slower than in air (Borken et al., 2006). Higher soil temperature in May and June compared to September may have increased CH_4 oxidation rates. Methane consumption increases when soil is dry and warm (Price et al., 2003). The Pearson correlations between soil

temperature and CH_4 oxidation rates confirmed this effect. Negative values showed CH_4 consumption was enhanced (became more negative) with the increase in soil temperature that occurred during the late spring and summer months. Some studies have shown that mineral N fertilizer application may decrease CH_4 consumption by an immediate inhibition of methanotrophs or change in the microbial community due to repeated applications (Bodelier and Lananbroek, 2004). In this sense, despite the positive correlations found between N-NH_4^+ and CH_4 comsumption values in the zero N and AN plots, there was no evidence of inhibition in this study.

4.4. Fluxes of CO_2

The CO_2 fluxes resulting from the grassland and measured by using static and opaque chambers represent ecosystem respiration. The application of slurries to the soil provides available C for soil microorganism activity, increasing of the microbial activity and resulting in large CO_2 fluxes after slurry application (Fangueiro et al., 2007; Mejide et al., 2009; Rochette et al., 2000). However, in this experiment total CO_2 fluxes from soils fertilized with SL resulted in similar CO_2 fluxes from soils with zero N or amended with AN. These results suggest that soil CO_2 fluxes may have been induced by the native soil C contents (Kuzyakov and Bol, 2006), rather than by the C contained in the slurry, and/or that another factor could have limited microbial respiration, not C.

The unusual warm and dry conditions observed during the experiment affected the pattern of soil CO_2 fluxes as the Pearson correlations confirmed when relating soil temperature and moisture to soil CO_2 fluxes. The highest soil CO_2 fluxes following the first and second N applications coincided with high soil temperatures and dry soil conditions and contrasted with the low CO_2 rates observed after the third application, in autumn. The relationship between respiration and temperature has been reported in other studies. Mejide et al. (2009) observed in a barley field that the lowest CO_2 emissions occurred in late autumn and winter, when soil temperatures were below 10 °C. Hynšt et al. (2007), in a grassland affected by cattle impact in Czech Republic, and Jones et al. (2006), in a temperate grassland in Scotland, observed the opposite. They observed the highest CO_2 fluxes with warm temperatures. Soil moisture contents were also related to the CO_2 fluxes observed. Dry soil conditions observed after the first two applications resulted in greater CO_2 fluxes than those observed after the third fertilization when high frequency of rainfall events was registered. In this sense, high WFPS may have suppressed soil respiration after the third fertilization by reducing soil CO_2 diffusivity rates (Rochette et al., 1991) and also by enhancing anaerobiosis and, in consequence, reducing aerobic respiration (Ball et al., 1999).

5. Conclusions

The unusual climatic conditions observed in 2011 in the SW England affected the pattern of fluxes of N_2O, CO_2, CH_4 during the experiment. Nitrification was probably the main pathway for N_2O production under the dry soil conditions (WFPS below 60%) observed during the first two applications. Large rainfall inputs identified after the third fertilization gradually increased soil %WFPS to saturation and could have enhanced conditions for complete denitrification. This situation also resulted in low N_2O fluxes from the SL and AN treatments. However, AN showed higher cumulative N_2O emissions compared with SL after the third application, probably due to greater anaerobicity and the lower N availability in the SL plots. High losses of N via NH_3 volatilization could have contributed to the low N_2O losses from the SL treatment, although these were modelled and not measured directly. Plant offtake of the applied N with both types of fertilizer also reduced the soil N pool available for potential N_2O production, especially during the first two applications. Methane emissions were recorded only from the slurry applications and the increase of soil moisture enhanced methanogenic processes which occurred within 48 h after the application. Carbon dioxide fluxes were influenced by soil temperatures and soil moisture contents, with the highest fluxes in the driest period of this study (May–June). The atypical weather during this experiment in some sense offers an indication of the effect of future climate change, with decreases in the annual pattern of rainfall and increased temperatures in SW England, especially in late winter and early spring; all of which could result in a decrease of N_2O emissions.

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APPENDIX 2

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Nitrous oxide emissions from forage maize production on a Humic Cambisol fertilized with mineral fertilizer or slurries in Galicia, Spain

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ABSTRACT

We conducted a field experiment in Galicia (NW Spain) to quantify N_2O emissions derived from fertilization practices carried out by local farmers growing forage maize (*Zea mays* L.). Forage maize was cultivated in a silt loam soil during 2008 and 2009, in different locations each year. Nitrous oxide fluxes were monitored during the whole growing season after the application of the following treatments: no N application (CN); 125 kg N ha^{-1} NPK at sowing and 75 kg granulated urea at top dressing (MN); 200 kg N ha^{-1} injected cattle slurry (CS) and 200 kg N ha^{-1} injected pig slurry (PS), with both slurry treatments applied at sowing. We observed that although fertilization significantly increased losses of N as N_2O, the type of fertilizer did not significantly affect the total cumulative N_2O emissions in either year. This could have been due to the high native soil C content in both experimental sites. Total cumulative N_2O emissions from N fertilized treatments ranged from 19.8 to 20.5 kg N ha^{-1} in 2008 and 10.8 to 11.7 kg N ha^{-1} in 2009, with the period between sowing and top dressing being the largest contributor. Nitrification probably caused the N_2O fluxes observed in the days following the application of N fertilizers in May, however the largest losses of N_2O were observed at optimal soil conditions for denitrification. Variations in N_2O fluxes between crop seasons could be attributed, in the first instance, to more N_2 rather than N_2O production during 2009, a consequence of the larger soil water filled pore space (>80%WFPS) during most of the sampling period. Another cause was probably the periods of soil rewetting observed in 2008, which triggered the largest N_2O fluxes observed and contributed to approximately 40% of the total cumulative N_2O emissions. The use of slurries or mineral fertilizer resulted in similar yield scaled N_2O emissions in both years. At a target N rate of 200 kg N ha^{-1} these were in the range 1.18–1.23 kg N_2O-N Mg^{-1} DM in 2008 and 0.51–0.58 kg N_2O-N Mg^{-1} DM in 2009. This study highlights the need to increase the knowledge of initial soil N contents at the moment of the N application and the dynamics of soil organic matter mineralization to adapt N rates to efficiently meet crop demands, especially in the period between sowing and top dressing application when demands are small.

1. Introduction

Nitrous oxide (N_2O) is a potent greenhouse gas that also plays an important role in the depletion of stratospheric ozone (O_3). The global warming potential of N_2O is 298 times that of the CO_2 in a 100-year time horizon (IPCC, 2007). Agriculture is the source of 10–12% of global anthropogenic greenhouse gases (GHGs), of which 60% is N_2O (Smith et al., 2007). Consistent with this trend, Spanish agriculture contributed approximately 76–78% to the total national N_2O produced in 2009 and 2010, 67% of which is from soil (MAGRAMA, 2012).

The application of organic and mineral fertilizers plays an important role in determining N_2O fluxes from soils (Mosier et al., 1998) via production from nitrification, the biological oxidation of ammonium (NH_4^+-N) to nitrate (NO_3^--N), and denitrification, the reduction of NO_3^--N to N_2. The contribution of these processes to N_2O production is controlled by soil oxygen (O_2) availability, mainly driven by soil water content. Generally any production at water filled pore space (WFPS) values below 60% is attributed to nitrification (Bateman and Baggs, 2005) whereas denitrification is the dominant process when WFPS exceeds 60% (Davidson, 1991). Large numbers of studies have considered denitrification to be the main N_2O producing process under high soil moisture (Abbasi and Adams, 2000; Skiba and Ball, 2002), but N_2O fluxes can decrease when extremely anoxic conditions are generated in the soil (WFPS exceeds 80%) and N_2O is reduced to N_2 (Davidson, 1991). As Galicia is one of the wettest areas of Spain (>1000 mm $year^{-1}$), agricultural practices in this region may contribute greatly to anthropogenic N_2O emissions. Agriculture has great importance in Galicia, in the North West of Spain, especially forage maize production. It accounts for 76% (68,909 ha) of the total area of Spain dedicated to this crop (ESYRCE, 2013). This is because Galician agriculture is associated with the production and use of forage crops at the farm scale to maintain

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dairy cattle production systems. Maize irrigation is not required in Galicia due to the cool summers and high rainfall (39% annual rainfall is registered between May and October). However, the application of N based fertilizers is necessary to enhance crop yields. Slurries generated on Galician farms are commonly applied to avoid the cost of mineral fertilizers. Also, crops can take up N more efficiently from slurries due to the slow release of nutrients they contain. This characteristic of the slurry can also help to reduce environmental problems such as leaching and, if slurry is injected into the soil, the risk of losses of N from ammonia (NH_3) volatilization (Smith et al., 2000). However, the injection of the slurries could stimulate N_2O fluxes (Sistani et al., 2010; Wulf et al., 2002) as slurries contain easily decomposable organic carbon (C) which can trigger denitrification and also generate anaerobiosis in the soil by increasing biological O_2 demand (Rochette et al., 2000).

The aims of this study were to understand the dynamics of the N_2O fluxes during the maize growing season under Galician climatic conditions and determine the influence of the type of fertilizer (injected slurries and mineral fertilizers) typically applied by local Galician farmers on the resulting emissions. We also aimed to identify what form of fertilization is associated with low N_2O production and high yields. This study will report N_2O emissions and crop yields from the maize growing seasons of 2008 and 2009, and present emission factors scaled by crop yields.

2. Material and methods

2.1. Location

Measurements were carried out for two consecutive growing seasons (2008 and 2009) on forage maize fields (*Zea mays* L.), which were established in different locations at Mabegondo Agricultural Research Centre (CIAM-INGACAL), Abegondo, Galicia, Spain (43.2425°N, 8.2608°W). The climate is European Atlantic (Fernández et al., 2011) and Mediterranean humid according to the agrologic classification provided by Papadakis (1966). The mean annual temperature for the period 1998–2007 was 13.1 °C, and the mean minimum and maximum temperatures were recorded in February (2.9 °C) and August (25.4 °C), respectively. The mean rainfall for the same period was 1101 mm. The wettest month was October (177 mm) and the driest July (35 mm). The average rainfall registered during the forage maize growing season (average of the period May–September over the last 10 years) is 237 mm and the average temperature is 16.1 °C. The soil in both maize crop seasons was a Humic Cambisol with a silt loam texture in the top 10 cm. The soil properties measured in each location are shown in the Table 1.

2.2. Experimental design

The experimental plots were established in May in both years as a randomized plot design with three replicates of four treatments: control without N fertilizer (CN), mineral fertilizer as NPK (15:15:15) and urea 46% (MN), cattle slurry (CS) and pig slurry (PS). Plots were 42 m^2 in area (4.2 m × 10 m) for control and mineral fertilizer plots. The area in those plots fertilized with cattle and pig slurries was 88 m^2 (8.8 m × 10 m) to accommodate the 4.4 m application width of the slurry tanker used for the slurry injection.

Table 1
Properties of the upper 10 cm of the Humic Cambisol in the maize cropping sites studied.

Year/site	Sand	Silt	Clay[a]	Total C	Total N	C/N	pH	BD[b]
	%			% DM[c]				Mg m^{-3}
2008	25	55	20	3.27	0.22	10.38	5.35	1.28
2009	19	56	25	3.60	0.35	10.22	5.30	1.25

[a] USDA classification.
[b] BD: bulk density was calculated by using the empirical formula provided by Battilani et al. (1999).
[c] DM: dry matter

2.3. Fertilization and slurry analysis

Mineral fertilizer was applied by hand in two doses at a final rate of 200 kg N ha^{-1}: 125 kg N ha^{-1} as NPK 15:15:15 fertilizer (7% nitrate N content, NO_3^--N; 7% ammonium N content, NH_4^+-N) before sowing (1st application, on 22nd May 2008 and on 22nd May 2009) and 75 kg N ha^{-1} as urea 46% for the top dressing when the plant was 40 cm high (2nd application, on 22nd July 2008 and 6th July 2009). Cattle slurry was from a storage pit located at CIAM and the pig slurry from a storage pit located on a private farm. Slurries were injected 20–25 cm into the soil at a target rate of 200 kg N ha^{-1} using a Joskin EMB tank with 8400 l of capacity and 40 cm band spacing injection. A sample of each slurry was analysed for total N prior to application to calculate an application rate equivalent to 200 kg N ha^{-1}. Cattle slurry was finally spread at rates of 66 and 69 m^3 ha^{-1} and pig slurry at rates of 62.5 and 76 m^3 ha^{-1} in 2008 and 2009, respectively (see slurries characteristics in Table 2). Before sowing, all plots were amended with P and K mineral fertilizers at a final rate of 100 kg P_2O_5 ha^{-1} and 250 kg K_2O ha^{-1}.

Slurry density was determined in fresh samples using a hydrometer (Nahita, Navarra, Spain). The pH of the slurry was measured with a pH meter (Crison GLP 22, Barcelona, Spain) after adding to 25 g of slurry to 25 ml of distilled water and stirring for 30 min. Slurry dry matter (DM) was determined after 24 h oven-drying at 105 °C. Organic matter (OM) was analysed in dry and ground (<0.01 mm) samples using a thermogravimetric analyzer TGA-601 (LECO Corp., MI, USA). Analysis of N, P and K was determined in fresh samples using Kjeldahl digestion (AOAC, 2000). Total N was analysed by a colorimetric method (indophenol blue reaction) using a continuous flow analyzer AA III (Bran + Luebbe, Norderstedt, Germany). Total P was determined by the colorimetric reaction between orthophosphate and ammonium molydate to form phosphomolybdate blue and analysed by a UV–vis spectrophotometer (Thermo Scientific Evolution 201). Dry samples of slurry were analysed on a Leco TruSpec CN (Leco Corp., MI, USA) to determine total C contents. Slurry K, Ca, Mg, Na contents were determined by atomic absorption spectroscopy. Ammonium (NH_4^+-N) and nitrate (NO_3^--N) contents were extracted from fresh samples using 2 M KCl. The extracts obtained were analysed using a segmented flow analyser AA III (Bran + Luebbe, Norderstedt, Germany).

2.4. Sowing, harvesting and plant analysis

Maize (*Zea mays* L., variety DKC3745) was planted on 22nd May 2008 and 22nd May 2009, with a 75 cm and 14 cm row and plant spacing, respectively. Planting rate was approximately 90,000 seeds ha^{-1}. Plots were harvested on 16th October 2008 and 29th September 2009.

Table 2
Chemical composition of the slurries applied in 2008 and 2009.

Property	2008			2009	
	Units	Cattle	Pig	Cattle	Pig
Density	g cm^{-3}	1.01	1.01	1.04	1.01
DM	%	7.30	2.13	6.57	2.22
pH	–	8.2	8.3	8.4	8.4
NH_4^+-N	%FM[a]	0.087	0.168	0.115	0.162
NO_3^--N	%FM	0.000	0.000	0.000	0.000
OM	g kg^{-1}DM	814	633	769	623
N	g kg^{-1}DM	42.0	119.5	43.3	110.3
P	g kg^{-1}DM	8.6	30.4	9.2	27.8
K	g kg^{-1}DM	44.1	84.9	47.3	83.2
Ca	g kg^{-1}DM	23.1	35.2	19.8	28.2
Mg	g kg^{-1}DM	6.1	18.2	6.9	14.7
Na	g kg^{-1}DM	6.1	13.2	3.9	11.3

[a] FM; fresh weight.

At the optimal maturity stage (the milk line is half to two thirds down the kernel), two central lines per plot were selected. In each line, 10 plants in a stretch of 7 m were cut with a sickle to 10–15 cm from the root and weighed. Samples of 300 g were selected for analysis of the percentage dry matter (%DM), measured by oven-drying at 80 °C for 18 h until constant weight. Dry samples were ground to pass a sieve of 1 mm and scanned on a near infrared reflectance spectrophotometer, NIRS Systems 6500 (FOSS NIRS Systems, Inc., Silver Spring, Washington, USA) for analysis of crude protein (CP) at 2 nm intervals between 1100 and 2500 nm. Spectral data were processed using WINISI software (ISI, Port Matilda, PA).

2.5. Nitrogen use efficiency

Dry matter yield and crude protein contents were used to calculate N uptake in plant tissue by the following equation (Eq. (1)):

$$N\ uptake : DM \times CP/100/6.25 \quad (1)$$

where *DM* is the dry matter (kg ha^{-1}), *CP* is the crude protein content (in %DM) and 6.25 is the conversion factor of protein to N content (MacDonald et al., 2002).

Apparent nitrogen recoveries (*ANR*) and apparent nitrogen efficiency (*ANE*) were calculated for each N treatment by the following equations (Eqs. (2) and (3)):

$$ANR(\%) : 100 \times (N\ uptake\ fert – N\ uptake\ unfert)/N\ fertilizer \quad (2)$$

$$ANE\left(kg\ DM\ kg^{-1}N\right) : (DM\ fert – DM\ \ unfert)/N\ fertilizer \quad (3)$$

where *N uptake fert* and *N uptake unfert* denote the above-ground plant N uptakes in the fertilized and control plots (kg N ha^{-1}), respectively and *DM fert* and *DM unfert* are the yields in fertilized and control plots (Mg ha^{-1}), respectively.

2.6. Measurements of N_2O fluxes-chamber design and operation

Nitrous oxide fluxes were measured using the closed chamber technique (Ryden and Rolston, 1983). Chambers were cylinders of polyvinyl chloride (PVC) with a volume of 0.018 m^3 (height: 36 cm; diameter: 25 cm) with a lid fitted with a rubber septum as a sampling port. Two chambers per plot (i.e., six chambers per treatment) were placed between rows and left in the same position during the experiment. On 30th May 2008 and 5th June 2009, before the application of the herbicide (4 l ha^{-1} as Acetochlor 45% + Terbuthylazine 21.4% (Harness GTZ)) and the insecticide (1 l ha^{-1} as Clorpyrifos 46%), chambers were removed to facilitate spreading and to avoid chamber contamination. After these applications they were re-inserted into the soil in the same position and sampling restarted 24 h later to avoid the effect of disturbance on the soil-atmosphere-gas transfer (Parkin and Venterea, 2010).

2.7. Nitrous oxide fluxes

Soil N_2O fluxes were measured a total of 70 times between 22nd May and 16th October 2008 and 45 times between 22nd May and 25th September 2009, and always between 09:00 and 10:00 h, which is the period of time considered to be representative of the daily mean (Alves et al., 2012). During the growing seasons of 2008 and 2009 and after the N applications, gas samples were taken three or five times a week. Chambers were closed for 60 min (T_{60}), the period of time where the headspace N_2O accumulation is linear (Chadwick et al., 2014). After this, a sample of the chamber headspace was withdrawn through the rubber septum using a 10 ml polypropylene syringe fitted with a hypodermic needle. An ambient sample was also taken to provide background values for N_2O (Cardenas et al., 2010; Chadwick et al., 2014) and the time zero sample (T_0). Each sample was transferred into a pre-evacuated 10 ml vial for storage and analysis. A sample of 12 ml was injected to over-pressurize the vials, preventing a back-diffusion of air (Hyde et al., 2006). The N_2O concentration of the samples was analysed in the laboratory using a gas chromatograph Thermo Finnigan Trace gas chromatograph (GC 2000) fitted with an electron capture detector (ECD) at 330 °C and HP-Plot Q column (30 mm × 0.3 mm). Nitrous oxide fluxes were calculated using the following equation (Eq. (4)):

$$F = \rho \times V/A \times (Ct – C_0)/t \times (273.15/T) \quad (4)$$

where F is the N_2O flux (μg N_2O-N m^{-2} h^{-1}); ρ the gas density (1.26×10^9 μg m^{-3} N_2O-N) under STP conditions (273 K and 101,325 Pa); V and A are the volume (0.02 m^3) and area (0.0491 m^2) of the chamber; C_{60} and C_o (μm^3 m^{-3}) are the N_2O concentrations 60 min after chamber closure (T_{60}) and the ambient sample (T_0), respectively; t is the time of chamber closure in hours (T_{60}); and T is the air temperature (K) at the time of sampling. Nitrous oxide fluxes were transformed to daily fluxes (kg N_2O ha^{-1} day^{-1}) for the calculation of the cumulative N_2O emissions. Nitrous oxide fluxes for dates between samplings were interpolated and the cumulative emissions calculated using the trapezoidal method (Cardenas et al., 2010; Louro et al., 2013). In order to obtain the cumulative N_2O emissions and the standard error for each treatment, cumulative N_2O emissions per plot (2 chambers) were calculated and the values obtained from the tree plots averaged.

Based on the N-applications and because of the split application of mineral fertilizer, cumulative N_2O emissions were calculated in two periods within each cropping season. In 2008 periods were: '2008I' (22nd May–22nd July) and '2008II' (22nd July–16th October). In 2009: '2009I' (22nd May–6th July) and '2009II' (6th July–25th September) (see Fig. 1).

Total cumulative N_2O emissions from the control treatment were subtracted from the corresponding values in N fertilized treatments during the whole period of measurements, giving net total N_2O emissions as a response to the N applied for each N fertilizer in each cropping season. Emission factors (EFs) for each N fertilizer were calculated by dividing the net N_2O cumulative emissions by the total N fertilizer applied and expressing it as a percentage. The assessment of the impact of cropping practices on greenhouse gas (GHG) production per unit yield is important for maintaining high yields with minimal GHG emissions (van Groenigen et al., 2010). Yield scaled N_2O emissions were calculated by dividing the total N_2O cumulative emissions in each treatment by their resulted crop yields and expressing them in kg N_2O-N Mg^{-1} DM.

2.8. Soil sampling and analysis

Soil samples at 10 cm depth were collected throughout both cropping seasons for the analysis of mineral N contents (ammonium (NH_4^+-N) and nitrate (NO_3^--N)) and soil moisture. Soil NH_4^+-N and NO_3^--N were determined by extracting 100 g of fresh soil with 200 ml 1 M KCl. Extracts were analysed colorimetrically for NH_4^+-N and NO_3^--N soil contents using a segmented flow auto-analyser AA III (Bran + Luebbe, Norderstedt, Germany). Soil moisture content was determined gravimetrically after oven-drying at 105 °C for 24 h. Porosity was calculated from the bulk density (BD) at each site by assuming a particle density of 2.65 Mg m^{-3}. Water filled pore space (WFPS) was calculated by dividing the soil moisture content and BD by the porosity and expressing it as a percentage.

2.9. Meteorological data

Daily rainfall (mm) and daily mean air and soil (10 cm depth) temperatures (°C) were obtained from the weather station located at CIAM which includes a pluviometer (Thies Clima, Germany, model 5.4032.35.007) and air (Geonica, Spain, model STH-5031) and soil (Campbel Scientific Spain, model T-107) temperature probes. Monthly

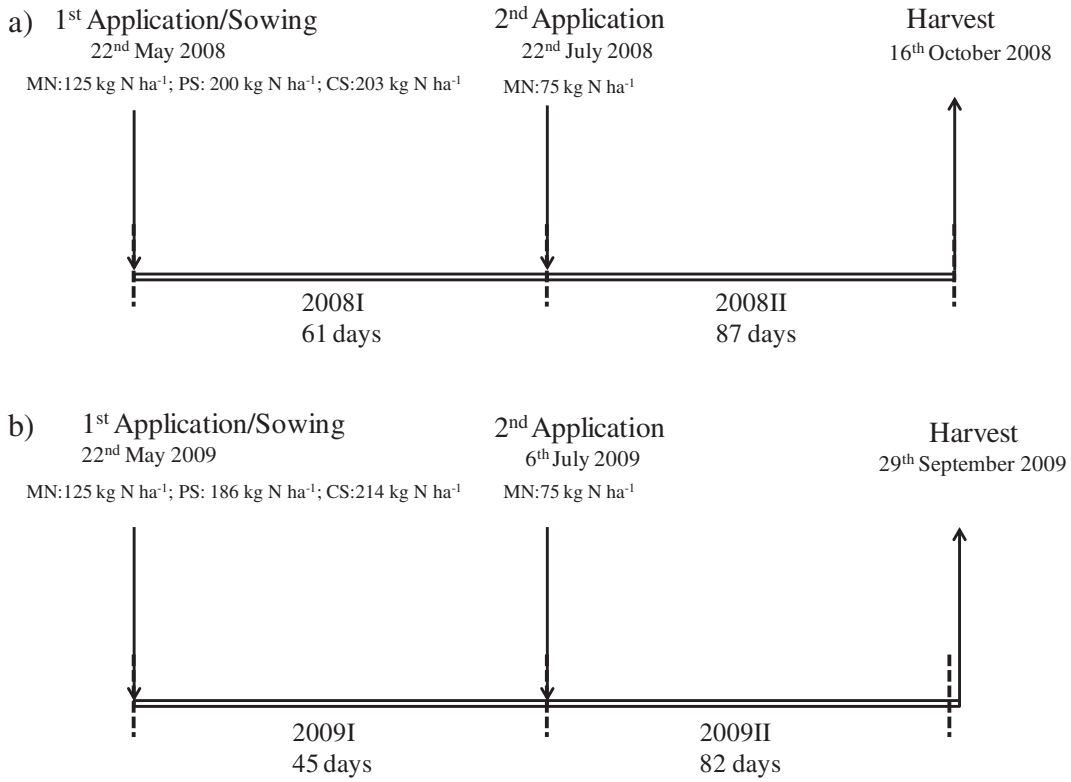

Fig. 1. Timeline and periods considered in a) 2008 and b) 2009.

mean rainfall and air temperatures for the last 10 years (1998–2007) and the corresponding means for 2008 and 2009 were also used.

2.10. Statistical methods

The statistical analyses were performed using Sigmaplot 11. Total cumulative N_2O emissions were transformed into mean cumulative emissions by dividing the total cumulative emissions by the number of days. This allowed comparisons to be made between periods and years that differed in numbers of days. Two way repeated measures analysis of variance (ANOVA) was used to determine significant differences between the periods within each year (i.e. periods '2008I' versus '2008II' and '2009I' versus '2009I') and between the years themselves (i.e. 2008 versus 2009). In each of these three analyses the treatment factor (CN, MN, CS and PS) was also included to determine a significant effect on the mean cumulative N_2O emissions. The same test was also performed to determine differences in EF within each year and between years. For total cumulative N_2O emissions, one way ANOVA was used to test for differences between treatments (CN, MN, CS and PS) within each period and also within each year. For WFPS and mineral N contents, two way repeated measures ANOVA was used to test for significant differences between treatments (CN, MN, CS and PS) and between soil sampling days within each period and year. For plant yield, N uptake, ANR, ANE and yield-scale N_2O emissions, one way ANOVA was used to investigate differences caused by the treatments (CN, MN, CS and PS). Tukey's test was used to determine which treatment groups were different at the $P \leq 0.05$ significance level.

3. Results

3.1. Weather conditions

Daily mean air temperature from the day of seeding to harvest was similar in both years, ranging from 12 to 25 °C in 2008 (Fig. 2a) and from 11 to 24 °C in 2009 (Fig. 2b). Daily mean soil temperatures were also similar with ranges of 13–22 °C in 2008 and 13—21 °C in 2009. Total rainfall during the sampling periods in 2008 and 2009 (Fig. 2a) was 360 mm (167 mm in '2008I' and 193 mm in '2008II') and 296 mm (145 in '2009I' and 151 mm in '2009II'), respectively. The first fertilization in both years was followed by high rainfall for the first 12 days in '2008I' and for the first 19 days in '2009I'. In '2008I', 11 days before the second fertilization, 28 mm of rain fell after a long period with no significant rainfall. The second N fertilization in both cropping seasons (periods '2008II' and '2009II') was not followed by large rainfall. In '2008II', large rainfall was observed between days 25 and 46; in '2009II' most of the rain fell in one single event on day 15.

3.2. Water filled pore space (WFPS)

The WFPS values reported in this study are approximate as they were derived using an estimated BD which was based on the sand and clay contents of the soil at each site (Battilani et al., 1999). We have carried out the statistical analysis of the treatments using this parameter but also provide the equivalent measured gravimetric soil moisture content (expressed as % water) as it is a more accurate descriptor of moisture for our study (Fig. 2c, d). No significant differences in soil WFPS between treatments were observed on any sampling day within either year ($P > 0.05$). Mean soil WFPS during '2008I' was 73% with the largest WFPS values in the following 25 days after the first fertilization (Fig. 2c). Also, in '2008I', the rain observed 11 days before the second fertilization increased soil moisture levels from approximately 50% to almost 60%WFPS. The beginning of the '2008II' period was characterized by dry conditions (as low as 30% WFPS) until the rainfall between days 25 and 46 increased WFPS to a mean value of 80%. In '2009I', the soil remained saturated (100% WFPS) or with standing water at the soil surface (WFPS > 100%) (Fig. 2d). In '2009II', throughout the period mean soil WFPS was 77% except for a couple of sampling days after day 15 where levels reached values >100%WFPS.

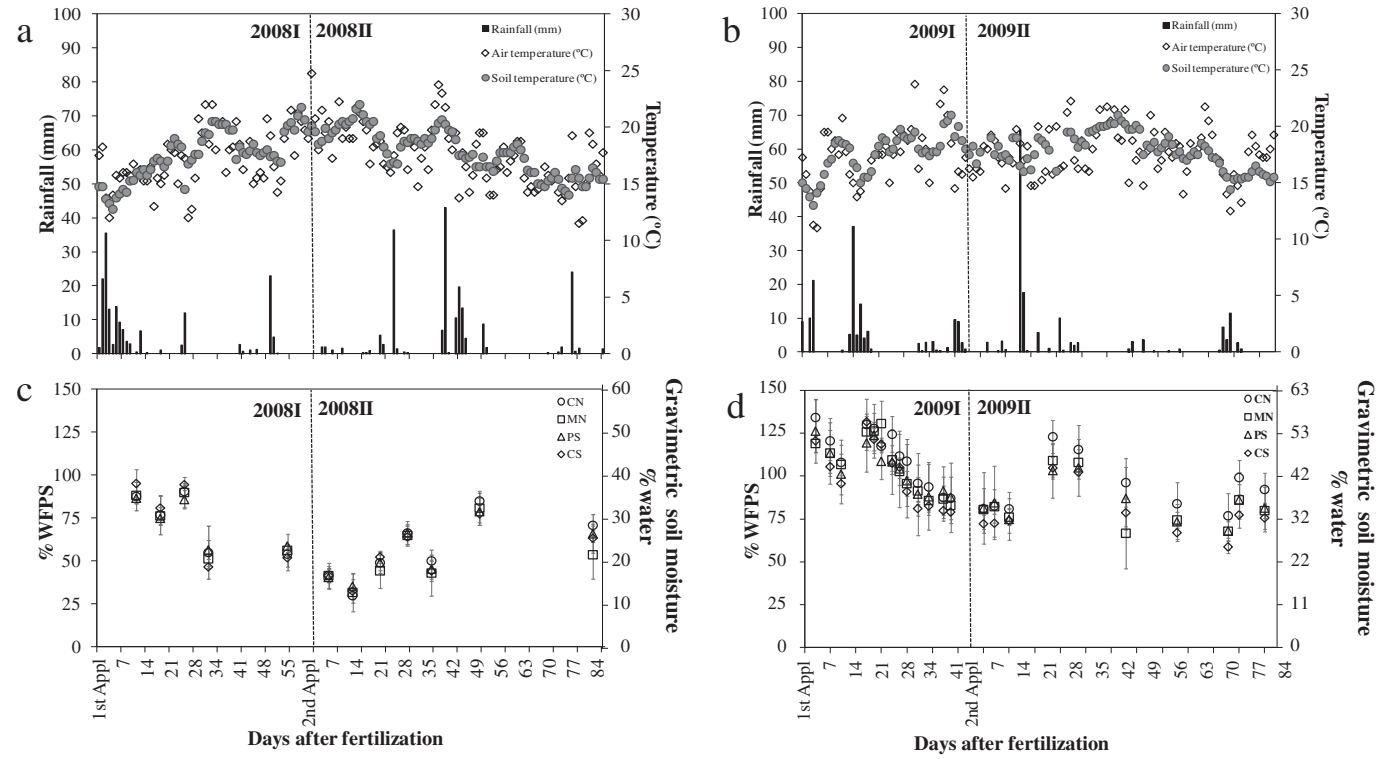

Fig. 2. Daily rainfall, soil temperatures and air temperatures in a) 2008 and b) 2009. Filled bars represent daily rainfall and unfilled and filled circles represent daily air and soil temperatures, respectively. Soil water filled pore space (%WFPS) and gravimetric soil moisture contents (%water) following fertilizations in c) 2008 and d) 2009. Data points and error bars represent mean value (n = 3) and standard error, respectively. Vertical lines mark periods between N applications. Treatments were: control (CN), mineral fertilizer as NPK + granular urea 46% (MN), injected pig slurry (PS), injected cattle slurry (CS). Periods were: '2008I' (22nd May–22nd July), '2008II' (22nd July–16th October), '2009I' (22nd May–6th July) and '2009II' (6th July–25th September).

3.3. Soil mineral N

3.3.1. Soil ammonium contents

In '2008I', soil NH_4^+-N levels (0–10 cm) in N-fertilized plots were not significantly higher as those in CN plots (Fig. 3a) ($P > 0.05$). In '2008II', NH_4^+-N values from MN plots were larger than the other treatments during the first 36 days ($P < 0.05$). In '2009I', significant differences between the N-treatments and the CN treatment were observed in the first 26 days ($P < 0.05$) (Fig. 3b). The largest soil NH_4^+-N contents were observed immediately after the first fertilization. In '2009II', soil

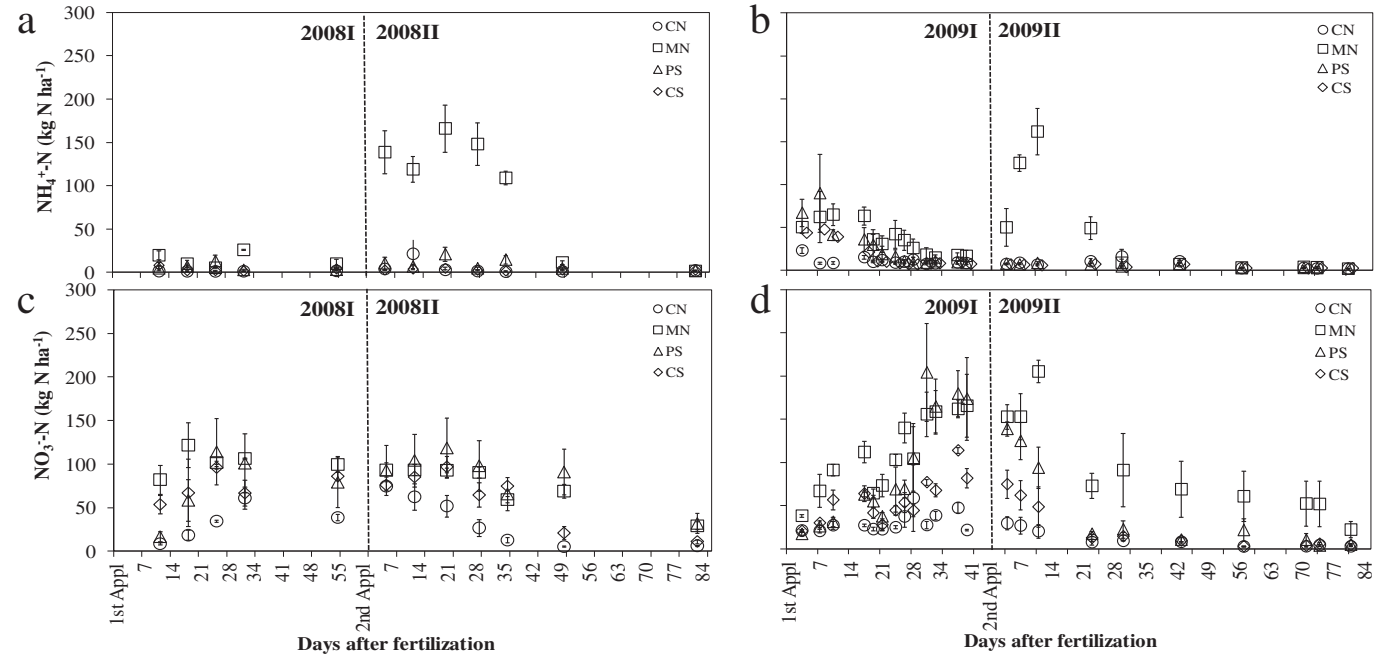

Fig. 3. Soil mineral N contents following N applications in 2008 and 2009. Soil NH_4^+-N in 2008 (a) and 2009 (b). Soil NO_3^--N contents in 2008 (c) and 2009 (d). Data points and error bars represent mean value (n = 3) and standard error. Vertical lines mark periods between applications. Treatments were: control (CN), mineral fertilizer as NPK + granular urea 46% (MN), injected cattle slurry (CS) and injected pig slurry (PS). Periods were: '2008I' (22nd May–22nd July), '2008II' (22nd July–16th October), '2009I' (22nd May–6th July) and '2009II' (6th July–25th September).

NH_4^+-N contents in MN plots were significantly larger than the other treatments during the first 23 days ($P < 0.05$).

3.3.2. Soil nitrate contents

In both years, soil NO_3^--N contents (0–10 cm) in the CN plots were in general lower than the other treatments ($P < 0.05$). However, short-lived increases in NO_3^--N levels from CN were observed in both years even when no N fertilizer was applied. During 2008, CN plots reached levels up to 61 kg N ha^{-1} on day 33 in '2008I' and 74 kg N ha^{-1} on day 6 in '2008II' (Fig. 3c). Maximum soil NO_3^--N contents of 97 and 118 kg N ha^{-1} were reached on day 21 in period '2008II' from CS and PS plots, respectively. In MN plots, fertilizations resulted in maximum soil NO_3^--N contents of 121.5 kg N ha^{-1} (on day 18 in '2008I') and 93.1 kg N ha^{-1} (on day 13 in '2008II'). In 2009, the largest soil NO_3^--N contents observed in CS and PS plots (114 and 204 kg N ha^{-1}, respectively) were reached on days 31 and 38 in '2009I', respectively. In the MN plots, although a similar pattern to the CS and PS plots was observed, the maximum soil NO_3^--N content (205.2 kg N ha^{-1}) was observed on day 11 in period '2009II'.

3.4. Nitrous oxide fluxes

In 2008, N_2O fluxes were large and frequent, even from CN plots (Fig. 4a). In '2008I', the largest N_2O fluxes were observed between days 51 and 55 from N-fertilizer treatments (1933, 2035, 1647 μg N_2O-N m^{-2} h^{-1} in MN, CS and PS, respectively) and on day 41 from CN plots (1137 μg N_2O-N m^{-2} h^{-1}). The largest N_2O peaks of the experiment from all treatments (range of 1772–2313 μg N_2O-N m^{-2} h^{-1}) were observed between days 27 and 41 in period '2008II'. In 2009, N_2O fluxes were less frequent and lower than in 2008. The largest N_2O fluxes in all treatments (range of 939–1642 μg N_2O-N m^{-2} h^{-1}) were reached on day 19 in '2009I'. In '2009II', the largest N_2O fluxes from the treatments (range of 751–992 μg N_2O-N m^{-2} h^{-1}) were between days 28 and 32.

3.5. Cumulative N_2O emissions

As Table 3 shows, N-based fertilizers significantly increased total cumulative N_2O emissions compared to the CN plots ($P < 0.001$) in both years and in the periods studied within each year ($P < 0.001$) but no differences between the forms of N applied were observed ($P > 0.05$). The patterns of the cumulative N_2O emissions from the treatments were similar to those from CN until day 55 in '2008I' (Fig. 4c) and day 21 in '2009I'(Fig. 4d), when N applications progressively increased losses compared to CN. Comparing losses in the two periods studied within each year, treatments resulted in mean cumulative N_2O emissions 1.4-fold larger in '2008I' than in '2008II' and 2-fold greater in '2009I' than in '2009II' ($P < 0.001$). Comparing years, in 2009 all treatments showed significantly lower mean total cumulative N_2O emissions than the corresponding values in 2008 ($P < 0.001$). In terms of EF, no significant differences between treatments were observed within each year and between years ($P > 0.05$). Losses of N_2O in relation to the N applied to the forage maize were 1.81% (MN), 1.63% (PS) and 1.74% (CS) (mean of both years).

3.6. Forage maize production and N uptake

In 2008, the application of N fertilizers resulted in similar DM yields and crop N uptake compared to maize plots with no N fertilizer application ($P > 0.05$) (Table 4), with overall mean values of 16.4 Mg DM ha^{-1} and 163.5 kg N ha^{-1} for DM yield and N uptake, respectively. In 2009, DM yields and N uptake from N fertilized treatments were 1.5 and 2-fold larger than for the CN treatment ($P < 0.05$) but no significant differences in these parameters between N-fertilizers were observed. In

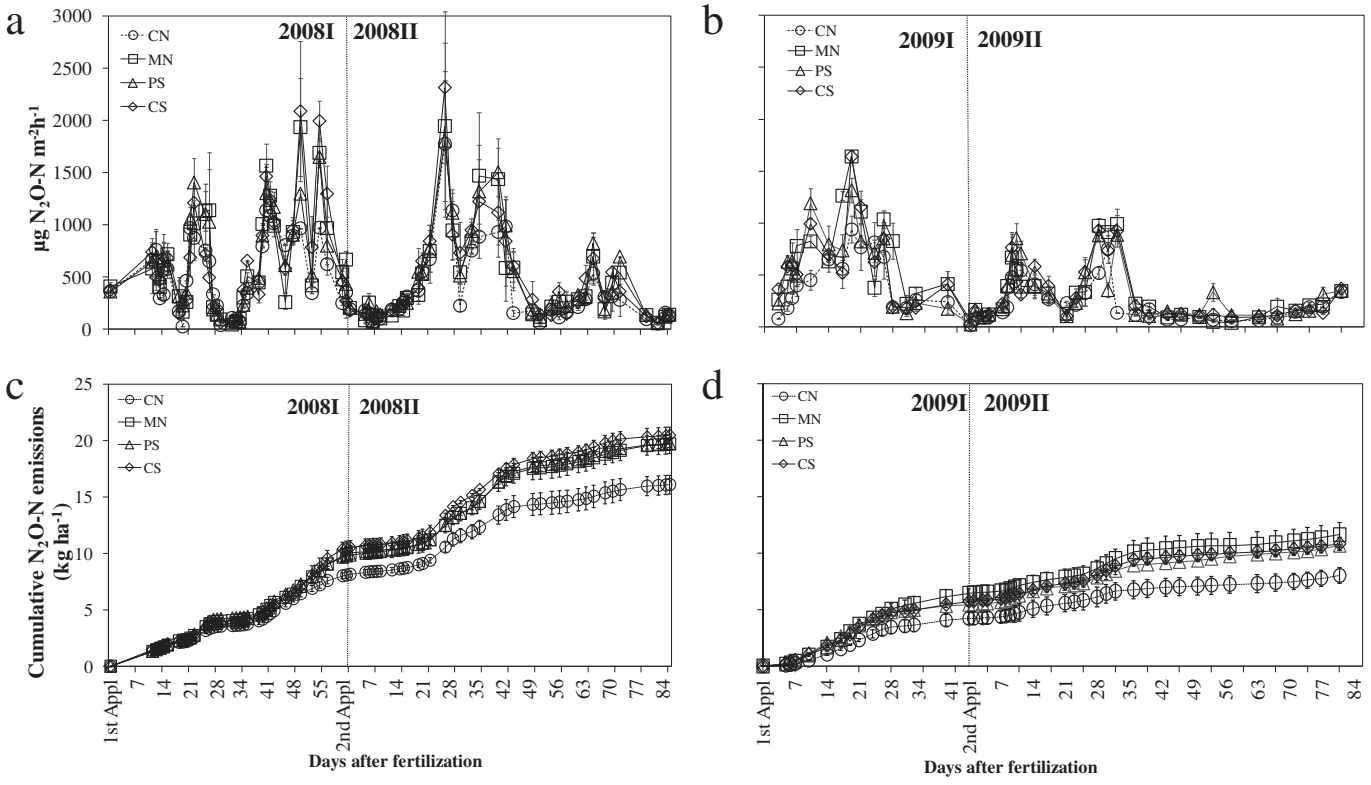

Fig. 4. Nitrous oxide fluxes after N fertilization in a) 2008 and b) 2009. Cumulative N_2O emissions in c) 2008 and d) 2009. Data points and error bars represent the mean value of three replicates and the standard error of the mean, respectively. Vertical lines mark periods between applications. Treatments were: control (CN), mineral fertilizer as NPK + granular urea 46% (MN), injected cattle slurry (CS) and injected pig slurry (PS). Periods were: '2008I' (22nd May–22nd July), '2008II' (22nd July–16th October), '2009I' (22nd May–6th July) and '2009II' (6th July–25th September).

Table 3
Nitrogen applied (kg N ha^{-1}), mean (kg N_2O-N ha^{-1} day^{-1}) and total (kg N_2O-N ha^{-1}) cumulative N_2O emissions in the periods studied after each N application and for the maize total growth season and the resulting emission factors in 2008 and 2009. Treatments were: control (CN), mineral fertilizer as NPK + granular urea 46% (MN), injected cattle slurry (CS) and injected pig slurry (PS). Mean values of three replicates and standard error between brackets. Treatments with different letters within each column and year are significantly different ($P < 0.05$).

Year/site	Treatment	'I'			'II'			Total ('I' + 'II')			
		N applied	Mean N_2O cumulative fluxes	Total N_2O cumulative fluxes	N applied	Mean N_2O cumulative fluxes	Total N_2O cumulative fluxes	Total N applied	Mean N_2O cumulative fluxes	Total N_2O cumulative fluxes	EF
2008		22nd May–22nd July 2008			22nd July–16th October 2008			22nd May–16th October 2008			
	CS	203	0.17 (0.01)[a]	10.5 (0.6)[a]	–	0.12 (0.01)[a]	10.0 (0.7)[a]	203	0.14 (0.00)[a]	20.5 (0.8)[a]	2.15 (0.53)[a]
	PS	200	0.16 (0.01)[a]	9.8 (0.4)[a]	–	0.12 (0.01)[a]	10.0 (0.8)[a]	200	0.13 (0.00)[a]	19.8 (0.4)[a]	1.82 (0.28)[a]
	MN	125	0.16 (0.01)[a]	9.9 (0.6)[a]	75	0.11 (0.00)[a]	9.9 (0.2)[a]	200	0.13 (0.00)[a]	19.8 (0.8)[a]	1.80 (0.07)[a]
	CN	–	0.13 (0.00)[b]	8.1 (0.3)[b]	–	0.09 (0.00)[b]	8.1 (0.3)[b]	–	0.11 (0.00)[b]	16.2 (0.8)[b]	–
2009		22nd May–6th July 2009			6th July–25th September 2009			22nd May–25th September			
	CS	214	0.13 (0.01)[a]	5.8 (0.2)[a]	–	0.06 (0.01)[a]	5.2 (0.4)[a]	214	0.09 (0.00)[a]	10.9 (0.4)[a]	1.33 (0.35)[a]
	PS	186	0.12 (0.00)[a]	5.5 (0.2)[a]	–	0.06 (0.00)[a]	5.3 (0.1)[a]	186	0.08 (0.00)[a]	10.8 (0.2)[a]	1.44 (0.35)[a]
	MN	125	0.14 (0.01)[a]	6.5 (0.4)[a]	75	0.06 (0.00)[a]	5.2 (0.3)[a]	200	0.09 (0.01)[a]	11.7 (0.7)[a]	1.82 (0.45)[a]
	CN	–	0.09 (0.01)[b]	4.2 (0.5)[b]	–	0.04 (0.00)[b]	3.8 (0.2)[b]	–	0.06 (0.00)[b]	8.1 (0.5)[b]	–

terms of ANE and ANR (Table 4), no significant differences between N-fertilized treatments were observed in any year ($P > 0.05$). However, ANE and ANR values in 2009 were 2.6 fold and 2.0-fold larger than the corresponding averages in 2008, respectively. The yield-scaled N_2O emissions (Table 4) showed no differences between N fertilized and CN plots in any year ($P > 0.05$) but values in 2009 were approximately 2-fold lower than those obtained in 2008 ($P < 0.05$).

4. Discussion

4.1. Influence of soil mineral N and moisture contents on N_2O fluxes

We assessed different N-fertilizers typically applied by local farmers during forage maize cultivation in order to identify the N fertilizer type that related to low N_2O emissions while maximizing production. However, the experimental sites had large initial mineral N contents in the soil profile (106 kg N ha^{-1} in 2008 and 123 kg N ha^{-1}, in 2009, data not shown). Low C/N residues from previous crops (pea/pea-triticale) were incorporated in both sites one year prior the maize season, followed by a fallow period during winter. So, the application of the different fertilizers at sowing further increased the mineral-N pool in the soil. This explains the high N_2O background values and those induced by the application of fertilizers observed throughout both cropping seasons. The losses of N_2O were especially high during the first period of each year e.g.'2008I' and '2009I' as the N plant requirements were low at this growth stage and soil WFPS and soil mineral N contents were high (Sehy et al., 2003; Tan et al., 2009; Drury et al., 2014). However, the total losses varied between years, probably due to the different soil WFPS (Fig. 2c, d). These differences could have also been related to the different positions on the slope of the experimental sites; at the bottom of a slope close to a river in 2009, and on the top in 2008. This would have resulted in large water contribution from the upslope land after rainfall episodes, causing saturated conditions and even standing water at the soil surface in 2009. Nitrification could have been the cause of the N_2O fluxes during the first days after the first fertilization in both years ('2008I', '2009I'). This is supported by the increase of soil NO_3^--N in all N treatments, especially in PS and CS where the N was applied entirely as NH_4^+-N (Fig. 3). However, this transformation was slower in '2009I' as soil mineral N was present both as NH_4^+-N and NO_3^--N ten days after fertilization where, at the same stage, mineral N was practically all as NO_3^--N in '2008I'. This was probably caused by the high soil WFPS contents in '2009I'. In addition, most of the existing NO_3^--N in MN plots and that already produced in CS and PS and also in MN plots during '2009I' could have been further reduced to N_2 (Smith et al., 2003) at such high soil WFPS levels, explaining the lower N_2O fluxes observed compared to '2008I' (Fig. 3). The CN plots also had an increase in soil NO_3^--N contents suggesting that mineralization of the soil OM and nitrification had occurred. The increase was larger in 2008 possibly due to the drying and wetting cycles observed during this year. During long periods of dry soil, a large fraction of the microbial population can die, increasing the levels of OM in the soil and, after soil rewetting, the surviving microbial biomass mineralizes that fresh OM (Van Gestel et al., 1993). Also these cycles could disrupt soil aggregates, exposing the large soil OM contents of this soil to a rapid mineralization (Goebel et al., 2005) and consequently emitting large pulses of N_2O, which would be in agreement with other studies carried out in temperate climates (Jørgensen et al., 1998; Kim et al., 2010), semi-arid climates (Barton et al., 2008; Dick et al., 2001) or even under laboratory incubations (Bergstermann et al., 2011). In our study, the resulting fluxes contributed approximately 40% to the total cumulative N_2O emissions from each treatment and denitrification would have been the largest contributor after both episodes of soil rewetting as soil NO_3^--N contents and

Table 4
Dry matter yield, N uptake, apparent N efficiency (ANE), apparent N recovery (ANR) and yield-scale N_2O emissions in the maize growth seasons of 2008 and 2009. Treatments were: control (CN), mineral fertilizer as NPK + granular urea 46% (MN), injected cattle slurry (CS), injected pig slurry (PS). Mean values of three replicates and standard error between brackets. Treatments with different letters within each column and year are significantly different ($P < 0.05$).

Year/site	Treatment	N applied	Yield	N uptake	ANE	ANR	Yield-scaled N_2O fluxes
		kg N ha^{-1}	Mg DM ha^{-1}	kg N ha^{-1}	kg DM kg^{-1} N	%	kg N_2O-N Mg^{-1} DM
2008	CS	203	18.3 (2.7)[a]	175.2 (17.7)[a]	19.65 (10.61)[a]	23.01 (4.70)[a]	1.18 (0.19)[a]
	PS	200	16.5 (1.2)[a]	177.0 (11.8)[a]	10.82 (3.98)[a]	22.12 (6.53)[a]	1.22 (0.09)[a]
	MN	200	16.4 (1.4)[a]	169.2 (9.8)[a]	10.27 (4.79)[a]	18.25 (3.16)[a]	1.23 (0.14)[a]
	CN	–	14.3 (0.5)[a]	132.7 (12.6)[a]	–	–	1.13 (0.07)[a]
2009	CS	214	20.6 (1.0)[a]	161.5 (10.8)[a]	32.58 (8.41)[a]	34.45 (8.86)[a]	0.53 (0.05)[a]
	PS	186	21.0 (0.4)[a]	176.1 (8.7)[a]	39.44 (5.79)[a]	47.48 (8.06)[a]	0.51 (0.01)[a]
	MN	200	20.2 (0.6)[a]	189.7 (10.3)[a]	32.93(3.73)[a]	50.93 (3.61)[a]	0.58 (0.05)[a]
	CN	–	13.7 (1.2)[b]	87.8 (13.2)[b]	–	–	0.59 (0.02)[a]

WFPS contents were not limiting for losses of N_2O by this pathway (Davidson et al. 1991).

4.2. Impact of mineral or organic fertilizer on cumulative N_2O emissions

Literature reviews including soils planted with maize have reported contradicting emission responses to the application of mineral and organic fertilizers (Table 5): greater N_2O emissions from mineral fertilizers in López-Fernández et al. (2007) and Chantigny et al. (2010) in clay soil, lower in Chantigny et al. (2010) in loam soil and van Groenigen et al. (2004). In agreement with our study, similar losses of N_2O from mineral and organic fertilizers are reported in Collins et al. (2011), Cai et al. (2012), Damdreville et al. (2008) and Meijide et al. (2007). The latter experiments, with the exception of those by Cai et al. (2012) and Meijide et al. (2007), corresponded to experimental sites with lower native soil C content but similar soil texture as in our experiment. The application of organic C contained in the slurries to soils with low C contents enhances microbial growth which promotes oxygen consumption and creates temporary anaerobic microsites (Cannavo et al., 2004), enhancing N_2O production by denitrification. Thus, the similar large N_2O emissions observed from mineral and slurry-treated plots in our experiment suggest that sufficient native soil C contents (Table 1) were available to support denitrification and that any differences between N_2O emissions were ultimately related to mineral N availability. This is also in agreement with the observations of Chantigny et al. (2010) and Pelster et al. (2012) in soils planted with maize and wheat, respectively, and both had high C contents.

The results reviewed in Table 5 confirm that the resulting EFs derived from maize cropping are not always similar the IPCC default value of 1% (IPCC, 2006). Under Mediterranean climatic conditions, even under lower soil C contents, urea or pig slurries under irrigation (López-Fernández et al., 2007; Meijide et al., 2007) resulted in EFs larger than this threshold value. In Canada (Chantigny et al., 2010; Gagnon et al., 2011), when optimal soil moisture conditions for N_2O losses by denitrification are combined with higher soil C contents than those in this study, the resulting EF are even higher. However, in silt loam soils (Collins et al., 2011; Damdreville et al., 2008, Table 5) fertilization caused similar or lower losses than 1%, contrary to our study, probably because of the lower soil C availability and greater soil aeration (WFPS 36.1–42.1% in Collins et al., 2011; 45–46%WFPS in Damdreville et al., 2008). This indicates that the IPCC default value understimates N_2O emissions from maize cropping in this region and in this type of soil.

4.3. Yield scale N_2O emissions

Expressing N_2O fluxes in terms of crop productivity did not reveal an association between fertilizer type, low N_2O production and high yields, possibly due to the large initial soil N contents in both soil sites. However, the results showed that even when similar types of fertilizers are applied at similar rates in the same soil type, yield scale factors vary largely from one year to other. In this sense, as it was mentioned, N mineralization could probably be more stimulated during 2008 because of the environmental conditions (e.g. soil moisture and temperature, Ma et al., 1999), increasing the amounts of mineral N available in the soil profile compared to in 2009. This resulted in the low efficiency of N fertilizers to increase yields during 2008, leaving larger quantities of N after harvest compared to 2009 (66–425 kg N ha^{-1} in 2008 versus 24–47 kg N ha^{-1} in 2009, data not shown). This indicates that the N inputs from mineralization varied seasonally, in agreement with Zhang et al. (2015) and Loecke et al. (2012) and thus, so do the optimal N rates for maize cropping as N demand changes (Olfs et al., 2005; Raun et al., 2009). So, the N_2O emissions observed during 2008 were related to

Table 5
Soil classification and properties (texture, soil OM, C contents), cumulative background and fertilizer induced N_2O emissions, and EF from derived from maize cropping studies reviewed.

Location	N applied (kg N ha^{-1}) (control/treat 1/treat 2)	Treatments[a] (control/treat 1/treat 2)	Soil classification	Soil texture	C content (g C kg^{-1})	OM (%)	Cumulative N_2O emissions (kg N_2O-N ha^{-1})			Emission factor (%)		Ref.[b]
							Control	Treat 1	Treat 2	Treat 1	Treat 2	
Madrid, Spain	0/170/170	Control/incorporated pig slurry/urea	Typic Xerofluvent	Sandy loam	8.2	1.4	2.91	5.08	5.89	1.27	1.80	1
Henan province, China	0/150/150	Control/organic manure/NPK	Aquic Inceptisol	Sandy loam	6.46	–	0.06	0.30	0.47	0.15	0.27	2
Quebec, Canada	0/100/100	Control/liquid swine manure/mineral fertilizer	Clayey, mixed, frigid, Typic Humaquept	Clay	46.8	–	2.63	7.10	11.61	3.1	6.6	3
Quebec, Canada	0/100/100	Control/liquid swine manure/mineral fertilizer	Loamy, mixed, frigid, Typic Dystrocryept	Loam	29.9	–	1.44	4.59	2.01	2.4	0.4	3
Wageningen, Netherlands	0/209/188	Control/cattle slurry/CAN	Typic Endoaquoll	Sandy	–	4	0.14	1.70	0.25	0.75	0.06	4
Wageningen, Netherlands	0/196/188	Control/cattle slurry/CAN	Typic Endoaquept	Heavy clay	–	6.4	1.52	3.37	2.75	0.88	0.66	4
Prosser, USA	0/336/336	Control/liquid manure/NPK	Coarse-silty, mixed, superactive, mesic Xeric Haplocambid	Silt loam	10	–	0.155	0.411	0.361	0.10	0.08	5
Rennes, France	0/110/180	Control/pig slurry/AN	Luvisol Redoxisol	Silt loam	9.5	–	1.00	1.028	2.167	1.07	0.02	6[c]
Rennes, France	0/180/132	Control/matured pig manure/AN	Luvisol Redoxisol	Silt loam	10.1	–	0.344	0.851	0.872	0.38	0.29	6[c]
Madrid, Spain	0/175/175	Control/pig slurry without DCD/urea	Typic Xerofluvent	Sandy loam	0.0089[d]	1.4	5.98	8.27	8.57	1.30	1.49	7
Quebec, Canada	0/200/200	Control/UAN/CAN	Fine, mixed, frigid Typic Humaquept	Clay	46	–	6.5	14.2	13.3	3.8	3.4	8

Note: in those experiments where different N rates of the same fertilizer were tested, the resulting N_2O emissions and EF after the application of similar N rates as in this experiment were selected. Also, in those works with more than one year of study testing the same N rate/s of the same fertilizer/s, the mean value of the N_2O emissions and EF obtained was considered.

[a] Treatments: CAN: calcium-ammonium nitrate; AN: ammonium nitrate; UAN: urea-ammonium nitrate; DCD: dicyandiamide.

[b] Ref.: 1, López-Fernández et al. (2007); 2, Cai et al. (2012); 3, Chantigny et al. (2010); 4, van Groenigen et al. (2004); 5, Collins et al. (2011); 6, Damdreville et al. (2008); 7, Meijide et al. (2007); 8, Gagnon et al. (2011).

[c] Calculated by lineal interpolation of periodic fluxes on the field.

[d] Expressed in g kg^{-1} of water soluble organic C.

the N rate that exceeded crop N uptake capacity (Snyder et al., 2009). Nitrogen fertilization strategies to maize cropping in this region must consider possible large N inputs from soil mineralization and adapt N fertilizer rates to match crop demands. Similarly, Hartmann et al. (2014) reported that N applications during maize growing season must be reduced as mineralization meets crop demands and N losses are very likely to occur. Also, they confirm that reductions in N application rates relative to conventional farming practices (excess of N inputs) do not affect maize yields and cause less N surpluses at harvest. Similar conclusions were also reported by Zhang et al. (2015). So, our study highlights the need to increase the knowledge of initial soil N contents at the moment of the N application and the dynamics of soil organic matter mineralization to adapt N rates to efficiently meet crop demands, especially in the period between sowing and top dressing application when demands are small.

5. Conclusions

We observed large differences in N_2O losses between the two maize cropping seasons studied even though they were performed in the same soil type. The different location of the sites could have influenced the contrasting soil moisture contents observed during both seasons and the extension of these events, which affected nitrification and denitrification processes. The objective of this study was to identify the N source that produced low N_2O production and high yields. However, we were not able to select one, as the forms of N applied caused similar N_2O losses to produce the same yields, probably due to the high C contents characteristic of the type of soil studied. It was also observed that the large initial soil mineral N contents at both sites, together with the application of the N treatments resulted in an accumulation in the soil after harvesting, especially in 2008. This study highlights the need to increase the knowledge of initial soil N contents at the moment of the N application and the dynamics of soil organic matter mineralization to adapt N rates to efficiently meet crop demands, especially in the period between sowing and top dressing application when demands are small.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.geodrs.2015.03.004. These data include Google map of the most important areas described in this article.

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